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WATER ANALYSIS

USER-FRIENDLY FIELD/LABORATORY MANUAL

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1996

**USAID FUNDED IFS-NAREPP/IRG PROJECT ON
QUALITY ASSESSMENT OF SURFACE WATER**

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Foreword

This User-friendly Field/Laboratory Manual for water analysis is a major contribution made by the scientists who are actively engaged in water-related research at the Department of Environmental Sciences, Institute of Fundamental Studies, Kandy. Successful effort has been made by them to compile cost-effective techniques for analysis of surface water. This simple but precise sampling techniques and analytical methods are quite appropriate for the researchers in this country. The unified format introduced in this manual for sampling techniques and analytical methods would make this an easy reference. In addition to sampling and analytical techniques, this manual also deals with other aspects of water quality assessment such as equipment maintenance, laboratory safety, quality control, quality assurance and dissemination of data. Diagnosis of errors in chemical analysis using basic statistics is also incorporated in this work.

Application of correct and relevant analytical techniques is essential in the implementation of programmes aimed at monitoring water quality. As shown in several previous studies, the analytical results become meaningful only if inter-laboratory comparisons are made. It is unlikely that this can be achieved without having an agreement for unified and similar methodologies. It is interesting to note that this aspect is also taken care of in this manual. This manual is undoubtedly a useful laboratory reference guide, especially in the context of Sri Lanka.

Professor Kirithi Tennakone
Acting Director, IFS.

Message

Observers of Sri Lanka's development process can scarcely ignore the fundamental contribution of water to the development process. Whether as a source of energy, an input to the production process, a medium for recreational activity or, in its most essential form, a source of life, water must be maintained in sufficient quality and quantity.

Because water is so important, society must monitor its use closely. The deterioration of water's quality and quantity - as evidenced through sickness in the population, contamination of ground water tables, or shortages in energy, for example- may have important social, economic and political repercussions.

Recognizing the importance of water quality monitoring, and the relative inaccessibility of timely and accurate water monitoring information, USAID and International Resources Group agreed in 1993 to give financial support to the Institute of Fundamental Studies to carry out a project on Quality Assessment of Surface Water in Sri Lanka. The work was to support, among other things, the production of a training manual on water analysis and assessment of the water quality of twelve selected water bodies in Sri Lanka. These two documents have now been completed.

NAREPP/IRG sees the production of these two documents as important steps in the longer process of improving water monitoring in Sri Lanka. The challenges remains significant, not least in beginning the actual monitoring of quality in the twelve identified water bodies. Additional challenges include clarification of the roles of the diverse institutions involved in water monitoring. It is our hope that the documents produced by the IFS may serve as input for those who aim, ultimately, to establish coordinated water quality monitoring systems and to support the institutions which can sustain them in the long run.

The Natural Resources and Environmental Policy Project (NAREPP/IRG)
International Resources Group, Prime Contractor
Colombo, 1996

Preface

Methodologies used in quality assessment of surface water change rapidly over time along with analytical advancements and subsequent technological inventions. Nonetheless, it takes time to accept a new technique for sampling or a method of analysis as being a standard method. Meaningful and object oriented water quality assessment programmes are launched at present for trend analysis. In the case of comparison, the reported data is valid only when similar methods are employed. Therefore, it is a prerequisite to have similar methodologies during sampling, processing and analysis when quality assessment programmes are launched by different laboratories. Different methods can be used in accordance with the availability of resources and cost-effectiveness. But techniques and methods should satisfy regional and global standards.

Careful examination of the available literature on surface water quality in Sri Lanka, in many instances revealed that the techniques used in sampling and the methods employed in analysis were not well- defined. Different methods have been employed to analyze the same constituent by different laboratories or individuals. The use of similar methods is extremely important when precision analysis is undertaken for constituents such as trace elements, micro-nutrients and organic residues.

The number of state institutions and the non-government and private sector organizations which are involved in water analysis in Sri Lanka shows a rapid increase today. In the process of quality assessment of surface water, outdoor sampling and bench work are always carried out by technical level field assistants and laboratory analysts respectively. It is quite clear that analytical techniques in many manuals are complicated with technical jargons and difficult to understand at a quick glance, especially for technical level analysts. Therefore, the validity of the data generated during such analysis is questionable. To fill the primary need for unified and similar methodologies in sampling and analysis and to make it convenient for technical level analysts, an attempt has been made to prepare this user-friendly field/laboratory manual as an activity component of the proposed project on quality assessment of surface water in Sri Lanka funded by the USAID and coordinated by NAREPP/IRG.

Sampling techniques and analytical methods described in this manual are not new but easy to employ and cost-effective. An attempt has also been made to simplify the text which describes the techniques and methods using a unified format and simple language. In this manual, priority is given only for sampling of surface water and analysis of important physico-chemical constituents and some bacteriological properties which are commonly used to assess water quality.

The rationale of water analysis and the principles and procedures underlined and the analytical techniques are first explained. The user maintenance of basic sampling equipment and laboratoryware is discussed next. Simple statistical methods to examine the precision and accuracy of results are described in Chapter 3. Chapter 4 of this manual has been entirely devoted to explaining the sampling strategies, the appropriate number of samples which should be taken for water analysis and field processes of sample treatment and storage. The way in which samples should be taken for microbiological examinations is also described in detail. All these sections will be of special interest to water analysts, scientists and to managers and administrators in the water industry. Analytical procedures of physical characteristics, chemical constituents and bacteriological properties described in detail in Chapter 5 will be of particular concern to water analysts. Attention is also drawn to the methods involved in laboratory organization, data processing, quality control and quality assurance. Finally, the interpretation of results and dissemination of data are discussed in the last chapter.

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GENERAL INTRODUCTION

Hydrological cycle is the cohesive link between three major components of the earth (i.e. biosphere, lithosphere and troposphere). Thus, water constitutes a continuum with different stages ranging from glacial ice to marine salt water. Lakes and rivers are considered as athalassic surface waters since they are confined to the land mass. In addition, there are several intermediate forms of surface water bodies which are either natural (e.g. estuaries, lagoons, flood plains, waterfalls, streams, brooks, creeks, pools etc.) or man-made (e.g. reservoirs, tanks, canals etc.) in origin.

Surface waters either natural or man-made are essentially solutions of chemicals of various concentrations and composition. Water also provides living habitats for aquatic organisms, from micro-organisms to marine mammals. The concentration of chemical constituents and the populations of aquatic organisms in natural waters vary depending on the type and the location of the water body. In general, chemical constituents and organisms found in natural clean waters are not harmful or detrimental to the organisms as well as human being. The nature and the type of constituents in water exhibit the quality of water. The quality of the aquatic environment shows temporal and spatial variations due to factors intrinsic and extrinsic to the water body. However, the quality of the water can be changed by man directly or indirectly by introducing substances or energy which results in deleterious effects on biodiversity, human health and beneficial uses.

Nowadays water in general, is not clean and therefore, not suitable for human consumption without prior treatment. Since, water is being contaminated with human wastes, agrochemicals, heavy metals or non-biodegradable organic compounds there is an urgent need for a prior assessment of surface waters for their physical properties, chemical constituents and biological communities.

The process of water quality assessment is an evaluation of the physical, chemical and biological nature of water in relation to natural quality, human effects and intended uses, particularly uses which may affect human health and aquatic biodiversity. Therefore, quality assessment of water provides basic information on the present status of water for determining trends in quality enabling the establishment of regulatory policies and implementation of mitigatory measures.

CHAPTER 1: ANALYTICAL TECHNIQUES: PRINCIPLES AND PROCEDURES

1.1 Introduction

All analytical techniques applied in water analysis are based on the measurement of a physical, chemical or a biological characteristic that is specific to the parameter sought for. The quantification procedure of the parameter, at one stage or another, depends on measuring the response of an instrument/equipment which is either simple or complex in function. Hence, a basic, yet thorough understanding of the principles involved in analytical techniques recommended for water analysis and instrumentation applied therein is a prerequisite for a water analyst in order to perform his task with confidence and to generate reliable as well as meaningful data that exhibit high accuracy and re-productibility.

Therefore, it was decided to furnish in Chapter 1 of this manual brief notes on major analytical techniques involved in water analysis. This chapter is divided into several sub headings each of which deals with a single analytical technique describing its principle, the instrumental design, technical aspects and the general experimental procedures. Critical points where errors can be introduced into analysis are also highlighted under the remarks.

1.2 Analytical Balance (weighing technique)

1.2.1 Principle

There are two types of analytical balances: mechanical and electronic. The conventional mechanical balance consists of a beam suspended on a knife edge and two weighing pans hanging on to the ends of the beam. The weighing is based on the phenomenon that the moment of force on the two sides of the center of gravity are equal at perfect balance. Normally the distances between the center of gravity and the points from which the two pans of the balance are hanging are equal. Thus, the weight of an object is straightforwardly given by the total weight of the standards placed in the other pan to counter balance it. The sensitivity of this balances normally ceases at 1 mg.

The more common mechanical balance is the single pan, semi-micro type of which generally has a weighing capacity of 100-200 g and sensitivity of 0.1 mg. In this balance, removable weights are attached to the pan side and the total weight of the pan side is counter balanced by a standard non-removable weight fixed to the other end of the beam. Thus, when an object is placed on the pan, the pan side becomes heavier and in order to bring the balance back to the previous position, some movable weights are removed from the pan side. Any

remaining imbalance is indicated on the optical scale of the balance. The weight of the object placed on the pan is given by the sum of the weights removed, plus the reading on the optical scale

The electronic balance operates on a more complex mechanism which is based on electromagnetic principle. Therefore, unlike in mechanical balances errors may occur when weighing materials with magnetic properties. Electronic balances are calibrated at the factory so that re-calibration may be necessary if the gravity in the laboratory differs considerably

1.2.2 Procedure

Basic steps involved in the weighing procedure of the electronic analytical balances are given below:

- Switch on the balance and allow sufficient time to warm up (usually 15 minutes).
- Calibrate the balance with the weighing chamber doors closed.
- Place the container (e.g. weighing boat, watch glass) on the pan and close the doors.
- Tare the container weight.
- Fill the container with the substance upto the targeted weight, close the door and record the weight.
- Remove the container filled with the sample and repeat the above three steps for the next weighings.
- Turn-off the switch after the weighings.

1.2.3. Remarks

- Locate the balance at a place where vibration is minimum (e.g. on a marble or a concrete slab) and is away from direct sunlight and large temperature fluctuations.
- Make sure to check whether the balance is in levelled position before making any measurement. If not, level it first.
- Always close the door of the weighing chamber before weighing.
- Never touch the weighing vessel or the pan with bare hands. Use forceps or a piece of good quality tissue to hold the vessel.
- Never put the material to be weighed directly on the weighing pan. Always use a container (i.e. weighing boat or watch glass).

1.3 Gravimetry

1.3.1 Definition of Symbols

Symbol	Definition	Unit
A	Analyte to be determined	--
P	Product (precipitate) formed by the chemical reaction between the analyte and the reagent	--
a/p	Molar (stoichiometric) ratio between the analyte and the product of the chemical reaction	--
[A]	Concentration of the analyte	mol l ⁻¹
W _p	Weight of the product	g
V	Sample volume used in the analysis	l
M _p	Molecular weight of the product	g

1.3.2 Principle

In gravimetry, the analyte of interest which is normally dissolved in a solvent is converted to a solid product (precipitate) of known chemical composition by means of a chemical reaction. The precipitate is then separated quantitatively from the solution and weighed after drying. The mass (quantity) of the analyte is calculated by means of the weight of the precipitate and the stoichiometry of the chemical reaction.

For the chemical reaction,



$$[A] = \frac{W_p}{V} \times \frac{1}{M_p} \times \frac{a}{p} \quad \text{mol l}^{-1}$$

1.3.3 General Procedure

The general procedure of gravimetric analysis includes steps given below. Analyte-specific procedures are given in the analytical procedures (see Chapter 5).

- Measure a known volume of the sample into a beaker.
- Add precipitate forming reagent as instructed in the relevant analytical procedure while mixing the solution.

- Filter the sample through a pre-weighed filter paper.
- Rinse the beaker with the filtrate several times and add to the filter paper.
- Thoroughly wash the precipitate with the same solvent as that of the sample, to remove impurities.
- Dry the precipitate to a constant weight in an oven maintained at the temperature specified in the analytical procedure.
- Cool the precipitate in a desiccator to room temperature.
- Weigh the precipitate by using an analytical balance.
- Calculate the concentration of the analyte using the weight of the precipitate and chemical data such as reaction stoichiometry and molecular weight as illustrated in section 1.3.2.

1.3.4 Remarks

- To improve formation of large crystals which facilitates easy filtration, add the reagent to the sample slowly with vigorous mixing. Also use a large volume of the sample for the analysis and maintain the temperature at an elevated level during the precipitate formation step.
- Keep the precipitate in the mother liquor at the elevated temperature (digestion) for a short period to improve the purity of the product and to promote the formation of large crystals. However, cool the solution to the room temperature (or to the temperature specified in the procedure) before filtering.
- Some gravimetric methods require the ignition of the precipitate prior to weighing in order to achieve a reproducible, stable and constant composition. In such cases, use ashless filter papers for separating the precipitate from the mother liquor.
- To minimize weighing errors, always bring the temperature of the weighing vessels containing the precipitate to room temperature in a desiccator before weighing. Do not touch the vessel by hand. Instead use a pair of tongs or forceps. While weighing, keep the glass doors of the analytical balance closed.

1.4 Volumetry

1.4.1 Definition of Symbols

Symbol	Definition	Unit
M_A	Molarity of analyte (A)	mol l^{-1}
M_T	Molarity of titrant (T)	mol l^{-1}
a/t	Stoichiometric (molar) ratio between A and T	--
V_A	Volume of the analyte sample used in the titration	l

V_1

Volume of the titrant used in the titration

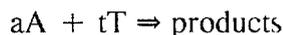
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1.4.2 Principle

Volumetry involves incremental addition of a reagent of known concentration to a fixed volume of a sample containing the analyte of interest until the reaction between the analyte and the reagent is complete. The completeness of the reaction is determined by an abrupt change in the colour of a third compound (indicator) added to the titration vessel at the beginning of the titration (note: other physical properties such as the chemical potential of solution can be used for the detection of the end point). As the indicator also has to react with the titrant to cause the change in physical property, the titration end point always surpasses the equivalent point causing a slight error in the reading (titration error).

In most of the instances, the titration error can be estimated by conducting a blank titration. Normally the estimated error is subtracted from sample titration data to improve the accuracy of the results. The quantity of the analyte in the sample is calculated using the titrant and sample volumes, molarity of the titrant and the stoichiometry of the chemical reaction pertaining to the titration.

e.g. for the general reaction,



$$\text{Moles of T at end point} = M_T V_T$$

$$\text{Moles of A at end point} = M_A V_A$$

$$M_A V_A = \frac{a}{t} M_T V_T$$

$$M_A = \frac{a}{t} \frac{M_T V_T}{V_A} \quad \text{mol l}^{-1}$$

1.4.3 General Procedure

The main steps involved in volumetric analysis are given below. Specific procedures may vary depending on the nature of chemical reaction between the analyte and the titrant and the physical property utilized for the detection of the end point. The analyst is therefore, requested to be familiar with the procedure that he intends to follow prior to analysis.

- Prepare a standard solution of the titrant by dissolving an accurate weight (use analytical balance for weighing) of a primary standard in a known quantity of distilled water. If the titrant is not a primary standard, standardize the solution against a primary standard.
- Rinse a clean burette with the titrant three times and fill it upto the top.
- Open the stopcock and remove all the air bubbles
- Take the initial reading to two decimal points (second decimal is your own estimate).
- Rinse a clean pipette with small portions of the sample three times and pipette a fixed volume of sample into a clean Erlenmeyer flask.
- Add few drops of the appropriate indicator to the flask and titrate with the reagent while swirling the flask.
- Wash down the walls of the flask with distilled water occasionally during the titration.
- When the end point approaches, add small increments from the burette until the slightest but the permanent change in colour results.
- Note down the final burette reading.
- Repeat the experiment at least twice and calculate the average analyte concentration using the equation given in section 1.4.2

1.4.4 Remarks

- Never rinse Erlenmeyer flask with the reagent or the sample to be pipetted in.
- Add only the recommended volume of the indicator to minimize titration error.
- Never try to bring liquid level of the burette down so as to coincide with a line of the burette as it is a useless and cumbersome endeavor.
- Never wash down the burette tip to the flask with distilled water. If there is a small droplet hanging onto the tip, deliver it into the flask by touching the tip on the inner wall of the flask and rinsing down the wall with distilled water
- Always run a blank titration to improve the accuracy of titration.

1.5 Potentiometry for pH Measurements

1.5.1 Definition of Symbols

Symbol	Definition	Unit
$[a_{H^+}]$	Hydrogen ion concentration of the sample	mol l^{-1}
pH	$\text{pH} = -\log[a_{H^+}]$	-
E_{cell}	Cell potential - potential difference across the H^+ sensitive (glass) membrane	V

C Constant

V

1.5.2 Principle

pH is a measure of hydrogen ion activity (however, exact activity cannot be measured due to interference of liquid junction potential). When a pH electrode is immersed in a solution, a potential difference is built up across the glass membrane due to difference in hydrogen ion activities of the internal and the external solutions. This potential difference is measured by two reference electrodes immersed in the two solutions and is related to hydrogen ion activity of the external solution (sample) as follows:

$$\text{at } 25^{\circ}\text{C, } pH = \frac{1}{0.0591} (E_{cell} - C)$$

1.5.3 Instrument Design

Basic components of a pH measuring system (i.e. pH meter) is given in Figure 1.1. The functions of these components are as follows:

pH electrode (combination type): The combination pH electrode consists of two components.

- a. A glass membrane (bulb) sensitive to hydrogen ion which is filled with a standard hydrogen ion solution (e.g. 0.1 M HCl) and consisting of a silver wire immersed in it as the internal reference electrode.
- b. An external reference electrode (e.g. Ag/AgCl electrode) fixed as the outer compartment of the electrode body which is connected to the external (sample) solution through a liquid junction (a membrane that permits the movements of ions).
(NB: In older models, pH (glass) electrode and reference electrode are found as two separate units).

Potentiometer (pH meter): This unit measures the potential difference across the glass membrane by means of internal and external reference electrodes, converts it into pH. It also consists of components needed for the calibration of the instrument (i.e. STANDBY, READ, CAL (calibration) and SLOPE knobs).

Stirrer: Stirring the sample solution improves the accuracy and the precision of pH measurement. Normally a separate magnetic stirrer is used for this purpose.

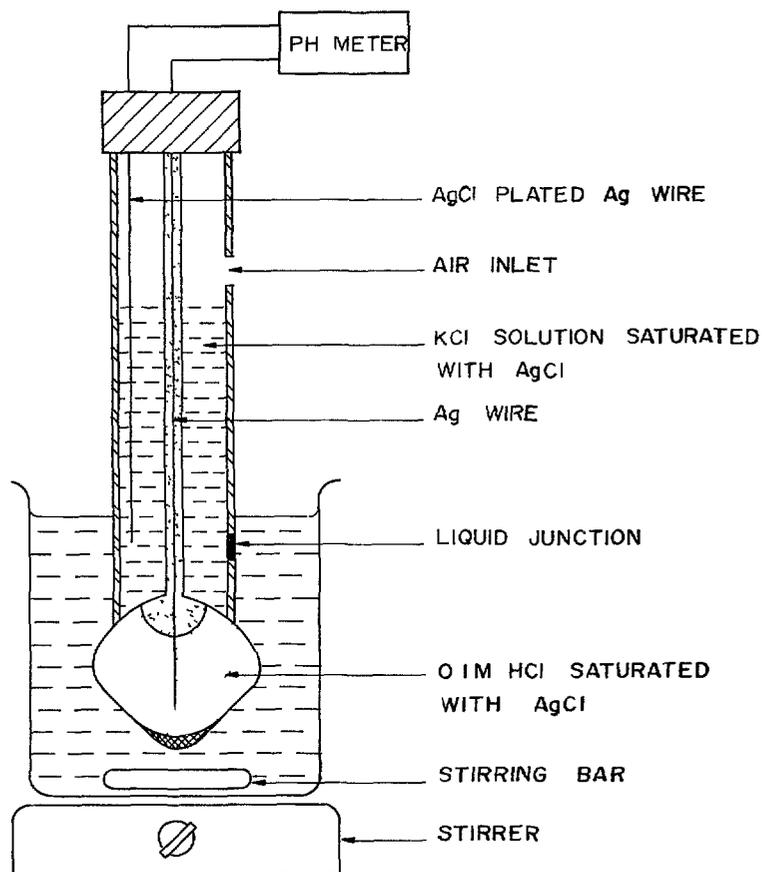


Figure 1.1 Schematic diagram of a pH measuring system

1.5.4 Calibration

The general procedure for calibration and sample pH measurement is given below. The analyst is, however, advised to refer the instruction manual of the pH meter at his disposal as specific procedure may vary depending on the make and the model.

- Check the filling solution level of the pH electrode (combination type) and if the level is low, fill in with the filling solution. If there are air bubbles inside, shake the electrode to remove them.
- Connect the pH electrode to the meter and remove the filling hole cap.

- Rinse the electrode with distilled water and shake off excess water. Blot the electrode dry with a tissue. Do not rub or wipe the electrode with any other material.
- Immerse the electrode in pH 7 buffer while stirring the solution and turn the meter to READ position
- Select temperature mode and measure the temperature of the buffer using built in temperature probe (if available) or measure it manually using a thermometer. Otherwise turn the pH meter to STANDBY position.
- Switch to pH mode.
- Turn the meter to READ position and wait for a stable reading.
- If the display value is different from the standard pH value for the measured temperature, adjust the reading with the CAL knob.
- Turn the meter back to STANDBY position.
- Remove the electrode, rinse it with distilled water and blot dry.
- Place the electrode in pH 4 buffer and stir the solution
- Turn to READ position and wait for a stable reading. Adjust the reading to the pH value corresponding to the measured temperature using SLOPE knob.
- Turn to STANDBY position, rinse the electrode with distilled water and blot dry.
- Place the electrode in pH 9 buffer and stir the solution.
- Turn to READ position and wait for a stable reading. If the pH reading is not between 8.9 and 9.1 the electrode requires cleaning in accordance with the procedure given in section 2.3.
- Place the electrode in the sample, turn to READ position of the meter and wait for a stable reading while stirring the sample.
- Record the pH and turn to STANDBY for the next measurement.
- After use, rinse the electrode with distilled water, blot dry and store it in electrode storage solution or pH 7 buffer

1.5.5 Remarks

- pH measurement is sensitive to changes in temperature. Therefore, calibration buffers and the samples should be at the same temperature. If they are different, use temperature compensation (as described in your manual) to minimize error in the reading.
- If the slope falls below about 90% during the calibration step or the meter readings in pH buffers drift with time, the electrode may have become contaminated. Then follow the cleaning procedure given in the section 2.3.1.
- Standard buffer solutions should be selected so that the range of the pH values expected in the samples is covered.

1.6 UV/Visible Spectrophotometry

1.6.1 Definition of Symbols

Symbol	Definition	Unit
P_0	Radiant power (incident) - energy of radiation impinging on one cm^2 of the sample	erg cm^2
P	Radiant power (transmitted) - energy of radiation transmitted from one cm^2 of the sample	erg cm^2
A	Absorbance ($A = \log P_0/P$)	--
T	Transmittance ($T = P/P_0$)	--
b	Path length (distance radiation travels through solution)	cm
c	Concentration of the radiation absorbing molecule	moll^{-1}
Σ	Molar absorptivity ($\Sigma = A/bc$)	$\text{mol}^{-1}\text{l cm}^{-1}$

1.6.2 Principle

When ultra violet/visible radiation travels through a solution, the power of incident radiation may be attenuated from P_0 to P as a result of absorption by molecular species. The attenuation of radiation, expressed in terms of absorbance is directly proportional to the path length (b) and the concentration of light absorbing molecule (c). Mathematically, it is expressed as

$$A = \Sigma bc$$

Where, Σ is molar absorptivity which is a constant for a particular analyte species at a given wavelength. This relationship is known as Beer's law. At constant path length (b), absorbance holds a linear relationship with the analyte concentration (c).

$$\text{Thus, } A = \Sigma bc = Kc$$

$$\text{where, } K = \Sigma b = \text{constant}$$

UV/Visible spectrophotometry is based on this fundamental relationship.

1.6.3 Instrument Design

Figure 1.2 depicts a single beam uv/visible spectrophotometer. The functions of the basic components of the instrument are as follows:

Radiation source: Generates uv/visible radiation by two sources
(1) deuterium lamp - uv range (190 - 350 nm)

(ii) tungsten lamp - visible range (350 - 850 nm)

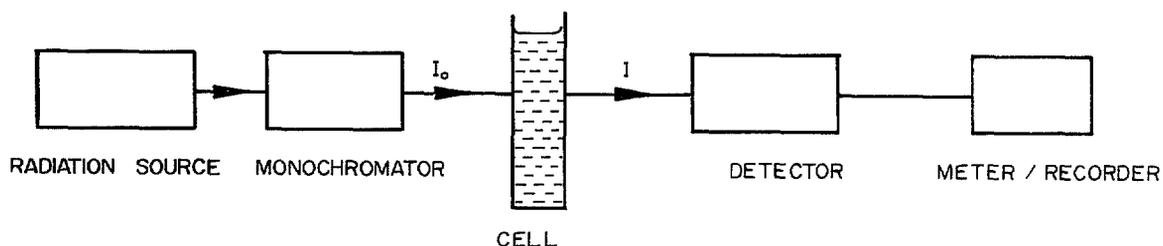


Figure 1.2 Schematic diagram of a single beam uv/visible spectrophotometer

Monochromator: Selects the wavelength of interest by means of a complex optical system.

Cell: Holds the standard or the sample of interest for absorption measurement. There are two types of cells (cuvettes) used in analysis depending on the wavelength.

- (i) quartz or fused silica cell: for ultraviolet region (below 350 nm)
- (ii) silicate glass or plastic cell: for visible region (above 350 nm)

Detector: Consists of a photocell system to measure the power of the radiation transmitted through the standard/sample.

Meter/Recorder: Displays the reading in analogue or digital form and records it (if recorder is available).

1.6.4 Calibration

Basic steps involved in the calibration of uv/visible spectrophotometer are given below. However, it is a requirement to read the manual of the instrument at your disposal before commencing measurements as calibration procedure may vary depending on the make and the model.

- Switch on the instrument and allow sufficient time (which is usually indicated in the manual, if not, 15-30 minutes) for warm up of optical and electronic systems.
- Select the desired wavelength (which is given in the analytical procedure of

interest). At this point, you may have to switch to the relevant radiation source manually.

- Fill up the cuvette with the blank solution upto 1 cm from the top, wipe the transparent surfaces with a soft tissue and insert in the cell compartment such that the light passes through transparent surfaces (caution: make sure to hold the cuvette by coarse surfaces only).
- Close the sample chamber lid and adjust absorbance to zero.
- Insert the cuvette(s) containing the standard(s) and record absorbances.
- Construct a calibration graph by plotting concentration of the standards against absorbance.
- Measure absorbance of the sample and calculate the analyte concentration from the graph.

1.6.5 Remarks

- The Beer's law is valid only for dilute solutions. Therefore, you must always prepare a calibration graph covering the concentration range of interest to verify the linearity.
- Spectrophotometric reading may vary with time due to electronic drift. To ensure accuracy in measurements, few points of the calibration graph should be re-checked occasionally during the analysis of samples. If there is a significant deviation, calibration graph should be corrected accordingly.

1.7 Atomic Absorption Spectrophotometry

1.7.1 Definition of Symbols

As of uv/visible spectrophotometry (Section 1.6.1).

1.7.2 Principle

A solution containing the analyte of interest is aspirated into a flame where the analyte absorbs incident radiation after atomization. The attenuation of the radiant power of incident radiation obeys Beer's law as described in Section 1.6.2

$$\text{i.e. } A = \Sigma bc$$

At constant path length $A = Kc$

where, $K = \text{constant}$

$c = \text{concentration}$

1.7.3 Instrument Design

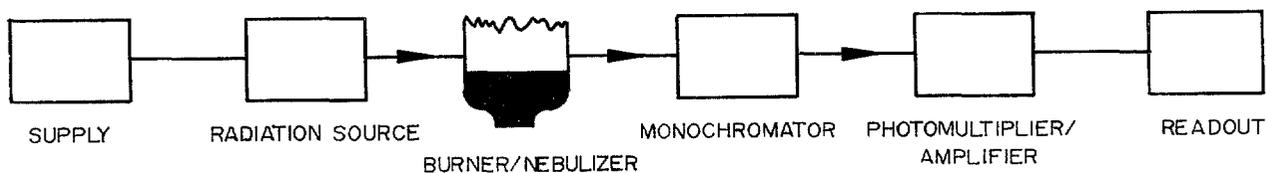


Figure 1.3 Schematic diagram of a single beam atomic absorption spectrophotometer (AAS)

Figure 1.3 depicts basic components of a single beam (flame) atomic absorption spectrophotometer. The functions of the main components are as follows.

Pulsed Power Supply: The power supply provides a high voltage to the radiation source in a particular pulse form.

Radiation Source: Widely used radiation source is hollow cathode lamp which consists of a cathode made of the element of interest and an anode made of tungsten. Pulsed power supply ionizes the filler gas and the positive ions formed during the process bombards on the cathode. This process generates a vapour of excited atoms of the element inside the lamp. During de-excitation of the atoms, a radiation characteristic of the element of interest is emitted. This radiation follows the same pulse pattern as of the power supply

Nebulizer/Burner : Nebulizer converts a liquid sample into fine aerosol and aspirates it into the flame through the burner. Generally, pre-mixed type burners are used where aerosols are mixed with the fuel and the oxidant gases first and only the fine droplets are allowed to enter the flame through the burner slot. In the flame, molecular species are vaporized and dissociated into elements. Part of the resulting atoms then gets excited by absorption of radiation receiving from the hollow cathode lamp of the same element. The atomization efficiency of a compound aspirated into the flame and therefore, the sensitivity of measurement depends on the temperature of the flame. Various fuel/oxidant combinations used in AAS to achieve a range of temperatures are shown below.

Fuel gas	Oxidant	Flame temp.
Hydrogen	Compressed air	2000 - 2100
Acetylene	Compressed air	2100 - 2400
Hydrogen	Oxygen	2550 - 2700
Acetylene	Nitrous oxide	2600 - 2800
Acetylene	Oxygen	3050 - 3150

Alternatively, electrothermal atomizers (graphite furnaces) can be used to improve the sensitivity of measurement.

Monochromator : Selects the wavelength characteristic to the element of interest and ensures that the photomultiplier receives only the radiation corresponding to that particular wavelength.

Photo-multiplier/Amplifier Photo-multiplier converts the optical signal into an electrical current. The amplifier enhances the current to a measurable level. The photo-multiplier is tuned to the pulse pattern generated by the power supply in order to distinguish between the radiation emitted from the hollow cathode lamp and that generated during de-excitation of the element in the flame.

Readout: Indicates the magnitude of the signal numerically either as a direct reading or as a printout, if a printer is available.

1.7.4 Calibration

The general operational procedure for calibrating AAS is summarized below. However, there may be model-specific differences in operational procedures of different instruments. Therefore, you are advised to be conversant with the operation of your instrument by carefully reading the manual before attempting any measurement.

Air/Acetylene Flame

- Make sure that the right burner head (i.e. air/acetylene) is installed, if not change the burner head.
- Make sure that the waste water tube is not folded or blocked and its end is submerged in water inside the plastic waste bottle.
- Load the hollow cathode lamp of interest into the turret of the instrument.
- Switch on the instrument and allow sufficient time for warm up of electronic and optical systems as specified in the manual, if not, 15 to

- 30 minutes.
- Set the lamp current and the slit width corresponding to the element of interest
 - Set the wavelength of the element of interest and optimize wavelength setting and lamp alignment
 - Turn on the fan of the exhaust system.
 - Open the gas flow of air and acetylene cylinders and adjust the desired gas flow rate of the instrument using flow meters (if compressed air is used as oxidant, turn on the air compressor and allow sufficient time to build up adequate pressure)
 - Ignite the flame and continue aspirating distilled water.
 - Select absorbance mode
 - Set desired background correction.
 - Aspirate the standard with the highest concentration and optimize absorbance reading by making burner adjustment.
 - Aspirate the reagent blank and set zero absorbance.
 - Aspirate standards and record absorbances.
 - Construct a calibration curve by plotting absorbance against concentration.
 - Aspirate samples and record absorbances.
 - Calculate analyte concentrations using the calibration curve
 - After use, aspirate distilled water for few minutes
 - Extinguish the flame by closing the fuel toggle
 - Close the fuel regulator at the cylinder and open the fuel toggle of the instrument to bleed the fuel gas completely and then shut it off
 - Turn-off the air compressor (or cylinder).
 - Turn-off the AAS and the exhaust fan

Nitrous Oxide/Acetylene Flame

- Install nitrous oxide/acetylene burner head.
- Perform the first nine steps of section 1.7.4
- Open nitrous oxide gas flow at the cylinder and adjust the desired gas flow rate using flow meter
- After burner head is warmed up for few minutes and if the flame is even, push the nitrous oxide button to change the oxidant from air to nitrous oxide.
- If necessary, make minor adjustments to the flame by changing gas flow rates.
- Perform analytical measurements following the next nine steps of section 1.7.4.
- Switch nitrous oxide gas flow back to air.

- Extinguish the flame by closing acetylene valve
- After closing acetylene and nitrous oxide regulators, open acetylene valve of the AAS until the fuel is drained off and then close it.
- Turn-off air compressor (or cylinder).
- Turn-off the AAS and the exhaust fan.

1.7.5 Remarks

- Beer's law is valid only for low concentrations. Therefore, the calibration curve should cover the concentration range of interest to verify the linearity.
- To ensure the accuracy of measurements, the drift of the instrument must be checked by re-analyzing a few standards occasionally during the analysis of samples. If there is a significant deviation in the readings, calibration curve should be corrected accordingly.
- Always be on alert about gas leaks (some models have built in gas leak checking facilities).
- Always maintain waste water level above the end of the waste water tube to avoid accidental fires.
- Make sure all gas supplies are closed when the instrument is not in use
- Always keep the exhaust system operating while the flame is on
- Replace acetylene cylinder when the pressure drops below 5.26 kgcm^{-2} (75 psi)
- Keep the area ventilated where AAS is installed and the gas cylinders (specially nitrous oxide) are stored
- Keep the gas lines free of oil to avoid spontaneous combustion or explosion
- When being used, heat the nitrous oxide pressure regulator to avoid freezing
- When the AAS flame is on, always keep the burner compartment door closed (otherwise wear tinted safety glasses).
- Do not use acetylene/nitrous oxide flame for the elements which are best determined by acetylene/air flame.
- If organic solvents are aspirated, discard the waste solvent collected in the bottle immediately after analysis.

1.8 Microbiological Characteristics

1.8.1 Introduction

Clean water is not a suitable substrate for the growth of micro-organisms. The microbial growth in water can be aggravated when water is contaminated with organic matter as it provides food for the microbes. Waste water usually contains a high level of micro-organisms (some of them are pathogenic) and these microbes can find their way into surface and ground water. Therefore, care

should be taken to make drinking water free of pathogenic microbes.

Microbiological analysis is used to determine the diversity and density of microbes available in natural and waste waters. In general, such analysis includes determining the total number of microbes capable of multiplying (total colony count) as well as detecting special classes of microbes which indicate the source of pollution (e.g. faecal pollution) and the micro biological characteristics of the micro-organisms (e.g. pathogenic). However, types of micro-organisms that can be found in natural and waste waters are given in Table 1.1.

Table 1.1 Types of micro-organisms that can be found in natural and waste waters

Type	Status
Bacteria	common
Viruses	rare
Yeasts	rare
Moulds	rare
Algae	common
Protozoa	common

Bacteria are the most important micro-organisms in water microbiology. Eventhough, considerably smaller in size than bacteria, the viruses are significant as far as the waste waters and surface waters are concerned because, they may be present to a more or less numerous extent. Analysis of water for viruses is time consuming and often troublesome and complicated. Therefore, determination of viruses are not carried out in the usual microbiological analysis. Yeasts and moulds are not very common in surface water and if present, they only play a subordinate role in surface water microbiology. They are frequently detected together with bacteria when analysis is carried out with culture media. Algae can also be considered as a group of microbes living in water. However, their diversity and density can easily be detected under light microscopes. The same applies to the unicellular animals known as protozoa. Bacteria are barely visible under an optical microscope with thousand fold magnification especially when the cells have been stained by a suitable method.

1.8.2 General Requirements for Microbiological Work

- Analysis of pathogenic microbes must be carried out only by experts having appropriate special knowledge, observing the necessary precautions.
- Analysis of non-pathogenic microbes also requires trained personnel and

- facilities that are dedicated for microbiological work.
- Glassware and equipment must be carefully cleaned and sterilized each time before they are used.
 - All apparatus must be mechanically cleaned using cleaning agents, rinsing first with clean tap water, then with 1% hydrochloric acid and finally with distilled or demineralized water. Glassware and equipment must be autoclaved at 120 °C for 30 minutes before the cleaning process to avoid infection in the process of washing and rinsing.
 - After cleaning, the apparatus and glassware must be first dried, then sterilized for 2 hours at 180-200 °C in a hot air sterilizer.
 - Culture media and culture solution must be sterilized with a superheated steam in an autoclave at 121 °C for 20-30 minutes at 1psi.
 - Secondary infections or technical errors in sampling can falsify the accuracy of the microbiological investigation. Therefore it is important that the sampling be carried out precisely.

1.8.3 Direct Method of Counting Micro-organisms

A counting chamber can be used to determine the number of bacteria in water. In such a chamber, the number of bacterial cells in a known small quantity of water (e.g. a cube with 0.001 mm³) can be counted under a light microscope. Each bacterial cell determined in such a cube (0.1 x 0.1 x 0.1 mm³) represents 10⁶ microbes in 1 ml. Thus, it is unlikely to arrive at reasonable estimate of microbes by this method if the microbial content is not very high in the cube. Direct counting can also be performed by filtering a known volume of water through a grided membrane filter paper (pore size 0.2 μm). The bacteria are dyed on the filter with appropriate dyes, the filter paper is illuminated and the counting of microbes can be performed by using a light microscope. This method certainly allows the low levels of bacteria when large quantities of water to be concentrated. However, the accuracy of this method is very low and it is also impossible to differentiate live and dead bacteria. In general, direct methods of counting bacteria are not employed in microbiological analysis of water.

1.8.4 Indirect Methods of Counting Micro-organisms

Indirect methods of counting microbes are usually practiced in microbiological analysis of water. In these methods, a known volume of water is added to a sterile culture medium which is then incubated under recommended conditions. After the incubation period, the colonies which have been formed are counted using a colony counter. It is assumed that each colony is a multiplication of a single living cell. The colony forming bacterial unit is either an individual cell

or a small accumulation of cells that was present in the water prior to the incubation period.

Standard value : In the case of disinfected drinking water, the maximum acceptable colony number should be 10 per 100 ml after the completion of the treatment process.

1.8.5 Indicator Micro-organisms

Drinking water must not contain living pathogenic bacteria such as *Salmonella*, *Shigella*, *Cholera vibrio* and other organisms capable of causing epidemics. The pathogenic bacteria are known as indicator microbes and can hardly be detected in normal cultures. The most important indicator microbes are the intestinal bacteria and the presence of these bacteria in surface water is an indication of faecal contamination. The microbes generally indicative of faecal contamination in water are *Escherichia coli (E coli)*, *Faecal streptococci* and sulphite reducing, anaerobic, spore-forming organisms. The coliform bacteria and *Pseudomonas aeruginosa* are used as indicator organisms to determine whether water is contaminated to a hygienically significant degree.

Since *E. coli* can only live for a short time in water, occurrence of this microbe in water indicates relatively fresh faecal contamination. Thus, when *E. coli* is present in water, one can assume the presence of other pathogenic bacteria (e.g. *Salmonella*, *Shigella* and *Cholera vibrio*) as well. *E. coli* can easily be coloured on media and be identified easily by checking its biochemical characteristics. It should be noted that the *E. coli* by itself is not pathogenic.

Standard value : In drinking water, *E coli* number must be zero per 1ml of water.

Coliform bacteria which belong to the family enterobacteria have the ability to ferment lactose forming gas and acid. Several types of coliform bacteria (e.g. *Enterobacter*, *Klebsiella* and *Citrobacter*) are frequently found in surface water contaminated with waste. They can also multiply outside the intestinal tract and are indicator organisms for hygienic significance of water quality.

Standard value : Coliform bacteria must not be detectable in drinking water.

1.8.6 Sampling

Generally containers used in the sampling of water for microbiological analysis are sterile, glass-stoppered bottles (250-500 ml) wrapped in aluminum foils. Bottles used for sampling chlorinated water must be treated with sodium thiosulfate before sterilization (for 250 ml bottle add 0.25 ml of 0.01 M sodium thiosulfate). The sample should be taken from approximately 30 cm below the surface. If no sampling device is available, a sample can be taken by immersing the sample bottle (after removing the aluminum foil) in water by means of a sterilized crucible tongs and slowly moving the bottle through the water approximately 20 to 30 cm below the water surface with the opening pointing diagonally upwards. Any contamination due to handling during this stage should be avoided. After filling, the bottle should be sealed immediately and the bottle neck should be protected with the aluminum foil.

1.8.7 Transportation and Storage

Sample bottles should be transported in insulated boxes without exposing to direct sunlight. Samples should be processed immediately after arrival at the laboratory. If this is not possible in exceptional circumstances, the sample should be stored at 4 °C. However, the period of storage should not exceed 24 hours. If the time interval between sampling and investigation is long, the bacteriological tests will have to be carried out *in situ*.

1.8.8 Methods for the Detection and Enumeration of Micro-organisms

As the number of indicator organisms in water may be very small, direct inoculation on solid media is not practicable and other methods must be used by which large volumes can be examined and by which the number of microbes in 100 ml of the sample can be estimated.

Methods which are commonly used:

1. Pour Plate Culture (Colony Count)
2. Multiple Test Tube (Most Probable Number: MPN)
3. Membrane Filtration

These techniques can be used to detect and enumerate each indicator organism, however, the media and the incubation conditions differing according to the organism sought:

1. Pour Plate Culture (Colony Count)

The usual method of counting heterotrophic bacteria in water is by pour-plate method with Yeast-Extract Agar. Separate counts are made of those

aerobic mesophilic micro-organisms which form visible colonies in this medium after 24 hours incubation at 37 °C and of those which form colonies after three days at 20-22 °C. The most useful application of the colony count is to detect change, especially sudden change in the microbial content of certain waters.

2. Multiple Test Tube Method (Most Probable Number: MPN)

In the multiple test tube method of counting bacteria, measured volumes of water or of one or more dilutions are added to a series of tubes containing a liquid differential medium. It is assumed that on incubation, each tube which received one or more test micro-organisms in the inoculum will show growth and the characteristic change produced by the micro-organisms sought when growing in the medium used. Provided that negative results occur in some of the tubes, the Most Probable Number (MPN) of micro-organisms in 100 ml of the sample can be estimated from the number and distribution of tubes showing a positive reaction. Confirmation that positive reactions are due to the growth of the specific indicator micro-organism sought can be obtained by subculture to tubes of confirmatory media, some of which need to be incubated at a higher temperature. Positive reactions in the confirmatory tests yield further information about the type of organisms present. The presumptive and confirmed counts are calculated by reference to probability tables.

3. Membrane Filtration Method

In this technique, a measured volume of the water sample is filtered through a membrane composed of cellulose esters. The pore-size is such that the micro-organisms to be enumerated are retained on the surface of the membrane which is then placed, (normally face-upwards) on a differential medium selective for the indicator micro-organism sought. This may be either an agar medium or an absorbent pad saturated with broth. On incubation at a selected temperature for a given time, it is assumed that the indicator micro-organisms, retained by the membrane will form colonies of characteristic morphology and colour depending on the medium used. The other micro-organisms are either inhibited or can be distinguished by their colonial appearance. The colonies of the micro-organisms sought are counted and the result is expressed as the number per 100 ml of the sample.

CHAPTER 2: USER MAINTENANCE: BASIC LABORATORYWARE

2.1 Introduction

Analytical instruments require constant attention of the user for them to function properly. Therefore, the instruction manual of any instrument contains a section on user maintenance. Proper care of instruments not only improve the sensitivity, accuracy and the reliability of the readings obtained by the instrument but also prolong their life time avoiding unnecessary expenses on new equipment.

This chapter describes, in general, the user maintenance procedures pertaining to the analytical instruments used in the water testing methods presented in this laboratory manual. The user is, however, strongly advised to read the relevant chapter in the instruction manual of the instrument before attempting any maintenance work. Also, note that maintenance of certain components of the instrument can be done only by the manufacturer or by an instrument specialist. The instruction manuals usually specify such components. Do not try any maintenance work of those parts.

2.2 Analytical Balance

- Whenever necessary, clean the weighing pan and the housing of the balance with some soapy water. Wipe out moisture completely with a clean and dry cloth.
- To remove any dust particles from the weighing chamber use a small artist's brush. Never blow air through the chamber

2.3 pH Electrode and Meter

Inspect the pH electrode regularly for scratches, cracks, salt crystal build ups or other visible deposits. If there are cracks or scratches replace the electrode. To remove salt or other deposits, follow the procedures given in sections 2.3.1 to 2.3.5. After cleaning the pH (combination type) electrode, remember to:

- drain the reference electrode solution, flush the chamber with new filling solution and refill.
- soak the electrode in storage solution for at least two hours.
- re-calibrate the electrode.

2.3.1 General Cleaning of the Electrode

- Soak the electrode in 0.1 M HCl or 0.1 M HNO₃ for half an hour and rinse

with distilled water.

- If unsuccessful, soak the electrode in a 1:10 dilution of household laundry bleach in a 0.1-0.5% liquid detergent solution prepared in hot water. Stir vigorously for 15 minutes. Rinse the electrode with distilled water.

2.3.2 Protein Removal

- Soak the electrode in 1% pepsin in 0.1 M HCl for few minutes to remove protein from the glass membrane or liquid junction. Rinse with distilled water.

2.3.3 Removal of Inorganics

- Rinse the electrode with 0.1 M tetra sodium EDTA solution and rinse with distilled water.

2.3.4 Removal of Grease and Oil

- Rinse with a mild detergent or methanol and rinse with distilled water.

2.3.5 Removal of Hard Deposits

- If all above cleaning procedures failed, immerse the electrode tip in 0.1 M NH_4F HF (ammonium bifluoride) for one minute and thoroughly rinse with distilled water. Check the slope of the electrode after refilling the reference electrolyte and soaking in storage solution for two hours. If the electrode slope is still unsatisfactory, discard the electrode.

2.3.6 Storage of Electrode

For short term storage (upto one week), store the electrode in storage solution supplied by the manufacturer or in pH 7 buffer/KCl system (1 g of KCl in 200 ml buffer)

For long term storage, discard the reference electrolyte, refill with fresh solution and cover the filling hole. Put few drops of storage solution into the protective cap and cover the electrode tip with it.

2.4 UV/Visible Spectrophotometer

- Keep the instrument in a clean dry place free from dust. When not in use, always keep the instrument disconnected from the main power supply and

covered with a dust cover.

- Clean cuvettes before and after use with high quality (analytical or HPLC grade) methanol and allow to dry. Never dry cuvettes in an oven or by exposing to a flame.

2.5 Atomic Absorption Spectrophotometer

2.5.1 Burner Cleaning

An uneven flame may indicate that the burner slot needs cleaning. If dilute solutions are aspirated the burner may require only occasional cleaning. If high concentrations of Ag, Cu and Hg salts are aspirated into air/acetylene flame, the mixing chamber of the burner requires immediate cleaning. After analyzing samples containing high levels of solids or those prepared in organic matrices (e.g. MIBK extraction) clean the burner immediately.

(a) General cleaning

- If uneven flame is noticed, turn-off the flame and carefully work along through the slot with a single edge razor blade. Be careful not to nick the edges of the slot. This will remove deposits lightly adhered to the burner slot.
- If the above method is unsuccessful, disconnect the burner head and carefully work through the slot with a razor blade. Remove scrapings from inside and outside the burner head.
- For further cleaning (if necessary) soak the burner head overnight in a detergent solution and rinse with deionized water and blow dry with a clean air flow.

(b) Cleaning after analysis of organic samples

- Aspirate for 5 minutes an organic solvent known to be miscible with the samples aspirated previously.
- Aspirate acetone for 5 minutes.
- Aspirate 1% HNO₃ for five minutes.
- Inspect the burner chamber and if deposits are to be seen clean the chamber using the cleaning procedure given in section 2.5.2.

2.5.2 Burner Mixing Chamber Cleaning

(a) General Cleaning

- Remove the burner head from the burner chamber and keep it aside.
- First, try to clean the burner chamber without dismantling it by pouring

- about 50 ml of water through the neck.
- If unsuccessful, remove the burner chamber from the instrument and dismantle it according to the instructions given in the manual.
 - Clean the burner head and the chamber with a detergent solution and a bottle brush. Do not use acid solutions or strong cleaning agents.
 - After cleaning, rinse thoroughly with deionized water and re-assemble the burner chamber.

- (b) Cleaning after use of high concentration of acetylide forming metals (e.g. As, Cu, Hg)
- Immediately after analyzing acetylide forming metals, thoroughly flush the burner mixing chamber with distilled water until all traces of residues are removed.

2.5.3 Cleaning the Nebulizer

A lower than expected reading in absorbance for a standard may be due to an obstruction in the nebulizer. Clean the nebulizer in the following manner.

- Aspirate pure water (or solvent) until absorbance reading is satisfactory for a subsequent standard.
- If it fails, insert a thin wire into the capillary from the inlet side and move it in and out.
- If the nebulizer path is still obstructed, disassemble the nebulizer according to manufacturer's instructions and clean the components with soap and water.

2.5.4 Care of Optics

- Do not make fingerprints on the surface of the hollow cathode lamp or on the light transmitting windows in the sample compartment of the instrument.
- When the covers of the instruments are removed, never touch the reflecting surfaces of mirrors and gratings.
- If dust gets collected on optical surfaces, blow it off carefully by using a clean and dry air flow. Do not rub the surfaces with a cloth.
- Window surfaces may be cleaned with a tuft of cotton moistened with a dilute solution of a mild liquid detergent followed by several rinsings with deionized water.
- Cleaning of mirror surfaces and the gratings should be done only by a skilled service engineer.

2.6 Glassware

2.6.1 Glassware Cleaning

The classical glassware cleaner is chromic acid solution which is prepared as follows:

Dissolve 100 g of $K_2Cr_2O_7$ in 1 l of distilled water. Add slowly 1 l of H_2SO_4 with great care while stirring. Allow the glassware to soak in chromic acid solution overnight. This mixture is hygroscopic and caustic so the container should be covered. Chromic acid is harmful and for many purposes, the safer modern preparatory glassware cleaner may be suitable. Modern glassware cleaners are complex chemical mixtures. Hence it is essential to make sure that they do not interfere with analysis. Ordinary detergents are less effective and more likely to cause interference. They may have disastrous effects on phosphorous analysis.

CHAPTER 3: STATISTICS IN WATER ANALYSIS

3.1 Introduction

Water laboratories worldwide generate daily, an enormous amount of information on water quality. This information contains quantitative values of chemical constituents and biological characteristics of surface or ground water. However, when report analytical data, basic questions arise are mainly on reliability and quality.

It is important to note that all measurements are associated with experimental errors upto a certain degree. In other words, it is impossible to achieve absolute precision and accuracy of the results. A knowledge on basic statistics helps us to estimate these experimental errors and to lay confidence on analytical data. An attempt is made in this chapter to brief the basic statistical calculations which should be performed routinely in laboratories which are involved in water quality assessment. The description will also include step-wise approach of calculation of statistical parameters from data and a discussion on its limitations. The use of these parameters in data quality control is also addressed

3.2 Basic Statistics Used in Data Analysis

3.2.1 Mean and Standard Deviation

Mean is the arithmetic average of all the measurements in a data set. The value of **standard deviation** indicates the spread of the individual measurements around the mean value. Small standard deviation signals very little variation of individual measurements around the mean.

Calculation

A. Mean (\bar{X})

$$\bar{X} = \frac{\sum_{i=1}^n x_i}{n}$$

where; x_i = i th measurement
 n = number of measurements

B. Standard Deviation (s)

$$s = \sqrt{\frac{\sum_1 (x_i - X)^2}{n-1}}$$

where: x_i = i th measurement
 X = mean of measurements
 n = number of measurements

The square of the standard deviation is called **variance** (s^2).

3.2.2 Comparison of Standard Deviation (Precision): F-test

The F-test is used to compare the standard deviations of two sets of measurements (*NB*: Standard deviation is directly related to precision. Therefore, by this way the precision of two sets of data can be compared).

Table 3.1 Values of F

Number of Degrees of Freedom of smaller variance	Number of Degrees of Freedom of larger variance										
	4	6	8	10	15	20	30	40	60	120	∞
4	9.60	9.20	8.98	8.84	8.66	8.56	8.46	8.41	8.36	8.31	8.26
6	6.23	5.82	5.60	5.46	5.27	5.17	5.07	5.01	4.96	4.90	4.85
8	5.05	4.65	4.43	4.30	4.10	4.00	3.89	3.84	3.78	3.73	3.67
10	4.47	4.07	3.85	3.72	3.52	3.42	3.31	3.26	3.20	3.14	3.08
15	3.80	3.41	3.20	3.06	2.86	2.76	2.64	2.59	2.52	2.46	2.40
20	3.51	3.13	2.91	2.77	2.57	2.46	2.35	2.29	2.22	2.16	2.09
30	3.25	2.87	2.65	2.51	2.31	2.20	2.07	2.01	1.94	1.87	1.79
40	3.13	2.74	2.53	2.39	2.18	2.07	1.94	1.88	1.80	1.72	1.64
60	3.01	2.63	2.41	2.27	2.06	1.94	1.82	1.74	1.67	1.58	1.48
120	2.89	2.52	2.30	2.16	1.95	1.82	1.69	1.61	1.53	1.43	1.31
∞	2.79	2.41	2.19	2.05	1.83	1.71	1.57	1.48	1.39	1.27	1.00

Calculation:

F-test

$$F_{\text{calculated}} = \frac{s_1^2}{s_2^2}$$

where, s_1 = standard deviation of data set 1
 s_2 = standard deviation of data set 2

$F_{\text{tabulated}}$ \Rightarrow obtained from the F-table for the degrees of freedom of data sets 1 and 2 and for 95% confidence limit (Table 3.1)

If $F_{\text{calculated}} > F_{\text{tabulated}} \Rightarrow$ no difference in standard deviations \Rightarrow no difference in precision.

- NB* *
- * Always put larger standard deviation value in the numerator of the formula
 - * Degree of freedom (n-1)

3.2.3 Comparison of Means (Student t-test)

Prior to applying t-test, it is required to perform the F-test in order to compare the standard deviations of the two data sets (the standard deviations of the samples should be same in order to compare means).

Calculation:

Case 1 When the true mean is known

$$t = \left[\frac{(X_1 - \mu)}{s_1} \right] n_1^{\frac{1}{2}}$$

where; X_1 = mean of data set 1
 μ = true mean
 s_1 = standard deviation of data set 1
 n_1 = number of observations in data set 1

Case 2

General case (with two means)

$$t = \frac{X_1 - X_2}{s_p \left(\frac{1}{n_1} + \frac{1}{n_2} \right)^{\frac{1}{2}}}$$

where;

$$s_p = \frac{[\sum_i (x_{1i} - X_1)^2 + \sum_j (x_{2j} - X_2)^2]}{[n_1 + n_2 - 2]^{\frac{1}{2}}}$$

where;

x_{1i}	=	ith measurement of data set 1
x_{2j}	=	jth measurement of data set 2
X_1	=	mean of data set 1
X_2	=	mean of data set 2
μ	=	true mean
s_p	=	pooled standard deviation
n_1	=	number of observations in data set 1
n_2	=	number of observations in data set 2

Confidence Interval

Both the mean and the standard deviation of a data set are only approximates. You may never reach the true value. By calculating **confidence interval** one can suggest a range in which the **true mean (μ)** resides. The probability (or the chance) level of the confidence interval should always be given.

Calculation

$$\mu = X \pm \frac{t s}{\sqrt{n}}$$

where;

μ	=	true mean
X	=	mean (calculated)
t	=	student t value (refer Table 3.2)
s	=	standard deviation
n	=	number of measurements

NB: * The true mean (μ) is called the population mean.
* (n-1) is called the degree of freedom.

Table 3.2 Values of Student's t-test

Degrees of freedom	Confidence level (%)				
	50	80	90	95	99
1	1.000	3.078	6.314	12.706	63.657
2	0.816	1.886	2.920	4.303	9.925
3	0.765	1.638	2.353	3.182	5.841
4	0.741	1.533	2.132	2.776	4.604
5	0.727	1.476	2.015	2.571	4.032
6	0.718	1.440	1.943	2.447	3.707
7	0.711	1.415	1.895	2.365	3.500
8	0.706	1.397	1.860	2.306	3.355
9	0.703	1.383	1.833	2.262	3.250
10	0.700	1.372	1.812	2.228	3.169
15	0.691	1.341	1.753	2.131	2.947
20	0.687	1.325	1.725	2.086	2.845
∞	0.674	1.282	1.645	1.960	2.576

3.2.4 Reliability of Results (Q-test)

Always reject the result of any analysis in which a known error has occurred. You may not, however, reject data arbitrarily. Do a Q test in order to make a decision on data rejection.

Calculation:

- To apply a Q-test, arrange the data of replicate analysis in the ascending order
- Calculate the **range** (the difference between the lowest and the highest value).
- Determine the **gap** (the difference between the questionable point and the value nearest to it)
- **Q = gap/range.**

- If $Q_{\text{calculated}} > Q_{\text{tabulated}}$ reject the questionable point.

Table 3.3 Q-table

Q(at 90% level)	0.94	0.76	0.64	0.56	0.51	0.47	0.44	0.41
n	3	4	5	6	7	8	9	10

3.2.5 Accuracy and Precision

The **true value** of a measurement always remains unknown. However, the values given for standard reference materials are accepted as true values. **Accuracy** is the nearness of a measurement or a result to the true value. It is not defined in statistics. However, by estimating the related term, **standard error** (σ_{μ}) of a series of measurements, you can get a feeling of the accuracy of the results.

Precision indicates how close the measurements are to each other. **Unlike accuracy, precision can be defined statistically.** It is obtained by calculating the standard deviation of a data set. For better indication of the precision of your data set, always calculate the related term, **coefficient of variation (CV).**

Calculation

a Standard Error

$$\sigma_{\mu} = \frac{X}{\sqrt{n}}$$

where: σ_{μ} = Standard error of mean
 X = mean
 n = number of measurements

b Standard Deviation

See section 3.2.1.

c Coefficient of Variation

$$CV (\%) = \frac{s}{X} \times 100$$

where: CV = coefficient of variation
 s = standard deviation
 X = mean of measurements

- NB:*
- * When the standard error of a data set gets smaller, the measurements become accurate (accuracy is high).
 - * When the coefficient of variation gets smaller, the measurements become more precise (precision is high).
 - * It is not possible to have accuracy without precision. However, you may have good precision without accuracy.

3.2.6 Rounding off Numbers

When you perform mathematical operations with your raw data (i.e. instrument readings) to determine the quantity of a desired parameter in the sample, you should maintain a certain number of digits in the answer in accordance with the rules of significant figures (rules governing significant figures are discussed in the next section). If your answer carries more digits than those allowed by the rules of significant figures, you should **round off** extra digits so that the rules are obeyed. For rounding off the numbers, you should follow the rules given below.

- (i) If the segment to be rounded off is more than half way to the next higher digit, add 1 to the last number to be retained.
e.g. If 3 numbers are to be retained,

	segment to be rounded off			
↓	↓	↓	↓	
67.0501	67.4501	67.5501	67.9501	
↓	↓	↓	↓	
67.1	67.5	67.6	68.0	

- (ii) If the segment to be rounded off is less than half way to the next higher digit, add 0 to the last number to be retained.
e.g. If 4 numbers are to be retained,

	segment to be rounded off			
↓	↓	↓	↓	
1.480499	1.482499	1.483499	1.489499	
↓	↓	↓	↓	
1.480	1.482	1.483	1.489	

- (iii) If the segment to be rounded off is exactly halfway to the next higher digit, round off the segment such that the last number to be retained becomes an even digit.
e.g. If 3 numbers are to be retained,

	segment to be rounded off			
↓	↓	↓	↓	
7.10500	7.13500	7.14500	7.19500	
↓	↓	↓	↓	
7.10	7.14	7.14	7.20	

3.2.7 Significant Figures

Let us consider a portion of a 50 ml burette as shown in Figure 3.1. On this burette, 1 ml segment is divided into 10 small graduations each representing 0.1 ml. Therefore, you can take the reading upto the first decimal point exactly. For example, any one can read the liquid level in the burette exactly as 36.4 ml. However, you may notice that you are able to estimate the second decimal place between the small graduations (between 36.4 and 36.5) visually. By doing so, you are improving the accuracy of your reading and the ultimate result of your titration/experiment. In this case, the second decimal place, of course, is not exact (i.e. contains some uncertainty). Different analysts may read the second decimal place differently.

You may take the above reading as 36.44 ml. Another analyst may read it as 36.43 or 36.45 or even 36.46 ml. Hence, the burette reading has at least ± 1 uncertainty in the last digit. Here, you cannot possibly take the reading upto the third decimal place. Therefore, a reading such as 36.442 deviates from accurate reading as the third decimal place (i.e. 2) is **insignificant** when taking the reading by this particular burette. You may now understand that the third decimal place is significant only if, in addition to 0.1 ml graduations, 0.01 ml graduations are marked on the burette as in the case of some micro burettes.

Now we can define the term **significant figures**. The number of significant figures in a reading obtained from an analytical instrument is the number of digits that are needed to express the reading without loss of accuracy. Therefore, a reading expressed in accordance with the concept of significant figures should contain all the certain digits and one uncertain digit. In the above example, 36.43, 36.44, 36.45 and 36.46 ml all contain correct significant figures (i.e. 4 figures) although the uncertainty in the last digit is different. Readings such as 36.4 ml and 36.442 ml do not comply with the concept of significant figures as the former does not include the first uncertain digit and the latter has one additional uncertain digit. If you are using a micro burette with 0.01 ml graduations, a reading like 36.442 ml obeys the concept of significant figures and therefore carries 5 significant figures.

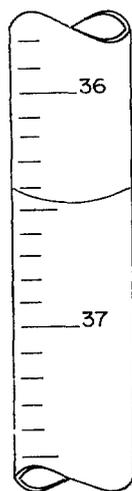


Figure 3.1 Segment of a burette

You must follow the same rules when taking readings from any instrument with an analogue or digital scale. In the case of digital scales, the digit next to the last stable digit of the reading (i.e. first uncertain figure) should be taken as the last significant figure of the reading.

You must also understand that sometimes you can find out the number of significant figures in a value, only if the value is reported in scientific notation. For example, the number 80,400 is ambiguous with regard to significant figures. You should write the number in scientific notation to find out number of significant figures. For instance above mentioned number possesses 3, 4 and 5 significant figures when expressed in different scientific notations

Number	Scientific notation	Measurement upto	Number of significant figures
80400	8.04×10^4	2 decimals	3
80400	8.040×10^4	3 decimals	4
80400	8.0400×10^4	4 decimals	5

3.2.8 Significance of Zero Digit in a Number

Let us consider the level of significance of the following numbers

Number	Scientific notation	Measurement upto	Number of significant figures
2 000102	2.000102×10^0	6 decimals	7
0.000102	1.02×10^{-4}	2 decimals	3
0.0001020	1.020×10^{-4}	3 decimals	4

You may understand from the above illustration that zeros are significant when they occur only in the middle of a number or at the end of a number on the right hand side of the decimal point. They are however, not significant when they appear before the first non zero digit on the left hand side of the number. Expression of the number in a scientific notation facilitates the deduction of the number of significant figures in it.

3.2.9 Mathematical Operations

You must follow the concept of significant figures throughout all mathematical operations that follow the initial experimental measurements. There are different rules governing addition and subtraction, multiplication and division, logarithm and antilogarithm.

(a) Addition and Subtraction

For addition and subtraction, express all the numbers by the same exponent and align them with respect to decimal point. Perform addition or subtraction and round off the answer so as to give the same number of decimal places as of the number with the fewest decimal places.

e.g

$$\begin{array}{rcl}
 (1) & 2.562 \times 10^5 & \\
 + & 1.035 \times 10^3 & \\
 + & 5.12 \times 10^6 & \Rightarrow \\
 \hline
 & & 5.377235 \times 10^6 \Rightarrow 5.38 \times 10^6
 \end{array}$$

$$\begin{array}{rcl}
 (2) & 8.015 \times 10^{-1} & \\
 & - 5.1 \times 10^{-3} & \Rightarrow \\
 & \hline
 & 7.964 \times 10^{-1} & \Rightarrow 7.964 \times 10^{-1} \\
 & \text{=====} & \\
 & &
 \end{array}$$

(b) Multiplication and Division

In these mathematical operations you should retain in your answer the number of digits contained in the number with the fewest significant figures. Here the exponent has no influence on the figure that is retained

$ \begin{array}{r} \text{e.g. } 5.46 \times 10^3 \\ \times 3 \\ \hline 16.38 \times 10^3 \\ \downarrow \\ 1.638 \times 10^4 \\ \downarrow \\ 2 \times 10^0 \end{array} $	$ \begin{array}{r} 4.3179 \times 10^{12} \\ \times 3.6 \times 10^{11} \\ \hline 15.54444 \times 10^7 \\ \downarrow \\ 1.554444 \times 10^4 \\ \downarrow \\ 1.6 \times 10^4 \end{array} $	$ \begin{array}{r} 1234.6 \\ - 2482 \\ \hline 497.4214343 \\ \downarrow \\ 4974214343 \times 10^2 \\ \downarrow \\ 4.974 \times 10^2 \end{array} $
---	--	---

(c) Logarithms and Antilogarithms

Consider the logarithm of number 584. Since the number contains 3 significant figures the logarithm value should also contain 3 significant figures.

$$\begin{array}{ccccccc}
 \text{e.g. } \log 584 & = & \log 5.84 \times 10^2 & = & 2.766412847 & = & 2.766 \\
 & & \downarrow & & \downarrow & & \downarrow \\
 & & 3 \text{ significant digits} & & 3 \text{ significant digits} & & 3 \text{ significant digits}
 \end{array}$$

Remember the first digit (2) in the answer is not significant. It only corresponds to the exponent in 5.84×10^2 . Therefore, here 2.766 (which is a log value) has only three significant figures. The rule is that the number of digits in the mantissa of a log value should be equal to the number of significant figures in the number of which the log value was taken.

In converting a logarithm to antilogarithm, number of figures in the mantissa of the logarithm should equal the significant figures in the antilogarithm value.

$$\begin{array}{ccccccc}
 \text{e.g. } \text{antilog}(-3.42) & = & 10^{-3.42} & = & 3.801893963 \times 10^{-4} & = & 3.8 \times 10^{-4} \\
 & & \downarrow & & \downarrow & & \downarrow \\
 & & 2 \text{ significant digits} & & 2 \text{ significant digits} & & 2 \text{ significant digits}
 \end{array}$$

3.2.10 Signal and Noise

Electronic signals will be produced by all equipment even when a blank sample is being analyzed. This electronic signal is called **noise**. The **Signal/Noise (S/N) ratio** is a measure of **equipment resolution**. See the manual of the particular equipment for this value.

Detection Limit (DL)

Detection limit is the concentration of an element which produces a signal to noise ratio of 2:1. The **detection limit** is the smallest concentration that can be detected from a given procedure with a given degree of confidence.

CHAPTER 4: SAMPLING, PROCESSING AND PRESERVATION

4.1 Introduction

Meaningful assessment of water quality depends on a variety of factors such as the design of monitoring programme, sampling, processing, pre-treatment, shipment to laboratory and the laboratory analysis. These are particularly crucial for some physical and chemical parameters such as, pH, trace elements, micro-nutrients (e.g. nitrate, phosphate, ammonia) and microbiological examinations (e.g. coliform counts, BOD₅). Errors can occur from field operations to laboratory analysis. Contamination is a common error that occurs during sampling, filtration and laboratory analysis. In the case of field measurements (e.g. pH, conductivity, temperature, etc.), uncalibrated operations will provide erroneous results. Understanding of hydrological regime is also fairly important to ensure meaningful field operations. Loss of labels and breakage of containers are also common sources of errors in the water quality assessment programmes.

It is also necessary to follow recommended procedures to avoid collection of non-representative samples. Each method or sampling gear (apparatus) has a specific procedure which should be followed accurately at every sampling occasion. In addition, simple basic rules such as avoiding unnecessary disturbances of the site prior to sampling (e.g. by standing in water and washing hands before sampling) must be followed. It is important to note that the selection of sampling procedures depend largely on the nature of the monitoring programme and the site to be sampled.

4.2 Sampling Strategies

Water sampling is fairly straightforward although certain factors must always be taken into account. For example:

- cleanliness of samplers and containers
- obtaining an adequate volume of samples
- collection of samples into special containers for certain analysis (e.g. determination of oxygen)
- filtration of samples in the case of dissolved constituent analysis
- quick shipment to the laboratory
- appropriate method of storage

4.3 Surface Water

True surface waterbodies in Sri Lanka are mainly rivers, estuaries and lagoons. Villus in the floodplain and marshes are also natural in form but transitional or seasonal in nature. It should be noted that Sri Lanka has no lakes. Reservoirs or irrigation tanks are anthropogenic in origin and are intermediate forms between rivers and lakes. Shallow irrigation tanks are fairly different from deeper highland reservoirs. Smaller irrigation tanks which are commonly known as village tanks are rain fed shallow pools. Several perennial pools can be seen in Hortan Plains, the highest altitudinal place of the country, of which the origin is unknown. A unique type of surface water in rock pools popularly known as "kema" is located in the southeastern part of the country.

4.4 Samplers

Popularly known water samplers (e.g. van Dorn, Ruttner, Kemmerer, Nansen) used in limnology are suitable for open waters and they are quite simple to operate. These samplers are made of polyethylene or polycarbonate material and are used for taking water samples at different depths with the aid of a messenger. van Dorn sampler, a horizontal type, is specially designed for taking water samples from desired depths (e.g. stratified sampling). However, all these samplers are expensive unless manufactured in-house.

Specific types of sampling bottles made of borosilicate glass are also available to sample water for chemical analysis (e.g. micro-nutrients, heavy metals, organic residues).

4.5 Sampling

4.5.1 Sampling for Physical Analysis

Samples for the analysis of physical parameters (i.e. temperature, conductivity, pH and oxidation/reduction potential) will seldom create problems. However, attention must be paid here to avoid changes that can be caused by entering or escape of gases.

4.5.2 Sampling for Chemical Analysis

The choice of bottle material and bottle size to suit the intended purposes of the analysis is important. Variable components such as Fe^{++} , cyanide, oxygen and free CO_2 must be determined at the time of sampling.

4.5.3 Sampling for Microbiological Analysis

Secondary infection or technical errors in sampling can falsify the accuracy of the entire microbiological investigation. It is therefore important that sampling be carried out expertly.

- NB:*
- * As a rule, sterilized glass-stoppered bottles covered with aluminum foils should be used to collect samples.
 - * Bottles used for sampling chlorinated water must be treated with sodium thiosulphate (0.25 ml of 0.01 M thiosulfate for 250 ml bottle) before sterilization.
 - * In the case of drinking water, taps must be initially cleaned mechanically and subsequently be flamed until they are completely dry.
 - * In order to prevent changes in microbiological quality of the water, samples must be transported in insulated boxes.
 - * The samples should be examined immediately on arrival at the laboratory. If this is not possible (in exceptional circumstances) the samples should be stored at 4 °C. However, storage time should be minimum.
 - * If the time span between sampling and investigation is too long the bacteriological tests will have to be carried out *in situ*.

4.6 Sample Treatment and Storage

Collected samples can be contaminated by inadequately or inappropriately cleaned glassware, filters, filter apparatus, chemicals used for preservation, etc. Thus, care should be taken in the cleaning of equipment and in the checking of the purity of chemicals used. Water quality parameters that should be determined in the field immediately after sampling need individual treatment so that these samples cannot be used for further analytical work. In addition, field analytical operations should follow a pre-determined sequence in order to avoid contamination. For example, conductivity must not be determined after measurement of pH in the same water sample because, concentrated electrolyte from the reference electrode used in the pH determination may enter the sample and affect the conductivity measurement.

During the field operations, periodic blank samples (one blank for every ten water samples) are required to determine errors arising from contamination. Usually for this purpose a distilled water sample is subjected to all the operations undertaken for the environmental sample such as filtration, storage and preservation. The blank is shipped with the other samples to the laboratory for analysis. When blank tests show evidence of contamination, additional investigations must be conducted during the next round of sampling.

Preservation of samples may be necessary in individual cases. The best form of preservation of a sample is the rapid investigation of the water sample after sampling. This should take place in the laboratory as soon as possible but not later than 2 days after sampling. During the transportation and until commencement of the investigation, the water sample should be stored in cool condition at 4 °C.

CHAPTER 5: ANALYTICAL PROCEDURES

5.1 Introduction

There are number of important points related to water analysis which are rarely mentioned in analytical manuals. Firstly, when a method is tried for the first time or when it is re-started after a break of some time, the precision (reproducibility) and the accuracy may be uncertain. Simply repeating the procedure without changes will often restore the earlier reliability. But it is important to assume that the first batch of measurements will not be satisfactory. It is also important to avoid discarding samples which cannot be obtained again.

Secondly, it should be a constant practice when a new standard solution is made, to check it against the old one. A sufficient amount of the old solution should be kept for comparison of the concentrations. This is necessary even when commercial standard solutions are used.

Thirdly, the analyst should constantly be seeking to match the precision and accuracy of his analysis to the needs of his problems and to the limitations of the sampling programme. It is usually waste of money to strive for accuracy of $\pm 0.1\%$ if the samples are unrepresentative or have the standard deviation of 10% or if the problem involves the comparison of two samples which differ in concentration by a factor of 10. Even within a single procedure there is a scope for judgement.

5.2 Physical Parameters

COLOUR

Spectrophotometric

Interferences:

Turbidity

1. Application

- 1.1 The method is applicable to potable, fresh and saline waters and effluents.

2. Sampling

- 2.1 Direct sampling

3. Preservation

- 3.1 Preserve at 4 °C

4 Apparatus

- 4.1 Spectrophotometer
- 4.2 Filtration system
 - 4.2.1 Filtration flasks, 250 ml with side tube
 - 4.2.2 Crucible holder
 - 4.2.3 Filter crucible
 - 4.2.4 Vacuum system

5 Reagents and chemicals

- 5.1 Filter aid- Celite No.505 or equivalent.

6. Procedure

- 6.1 Use two 50 ml samples, one at the original pH and the other at 7.6 pH, both at room temperature (use sulfuric acid or sodium hydroxide to adjust the pH).
- 6.2 Remove excessive quantities of suspended materials by centrifuging.
- 6.3 Treat each sample as follows.
 - 6.3.1 Thoroughly mix 0.1 g filter aid in a 10 ml portion of centrifuged sample and filter to form a precoat in the filter

- crucible.
- 6.3.2 Direct filtrate to waste flask.
 - 6.3.3 Mix another 40 mg of filter aid in a 35 ml portion of centrifuged sample.
 - 6.3.4 With vacuum still on, filter through the precoat and pass filtrate to waste flask until clear.
 - 6.3.5 Direct clear filtrate flow to clean flask and collect 25 ml for the transmittance measurement.
- 6.4 Thoroughly clean 1 cm absorption cell with detergent and rinse with distilled water.
 - 6.5 Rinse twice with filtered sample and fill the cell.
 - 6.6 Determine transmittance values at the wavelengths given in section 7.1.

7. Calculation

- 7.1 Express colour characteristics in terms of dominant wavelength (refer the Table 5.1).

Table 5.1. Wavelength range and corresponding colour

Wavelength Range (nm)	Colour
400-465	Violet
465-482	Blue
482-497	Blue-green
497-530	Green
530-575	Greenish yellow
575-580	Yellow
580-587	Yellowish orange
587-598	Orange
598-620	Orange-Red
620-700	Red

8. Alternative method

- Colorimetry with platinum/cobalt scale (USEPA, 1983 ; APHA, 1989)

NB: * Since biological activity may change the colour characteristics of a sample, the determination should be made as soon as possible.

TEMPERATURE °C (Celsius)

Thermometric

1. Application

- 1.1 The method is applicable to potable, fresh and saline waters and effluents.

2. Sampling

- 2.1 *In situ* measurement

3. Apparatus

- 3.1 Mercury thermometer calibrated in Celsius. The smallest scale of the thermometer should be 0.1 °C.

4 Reagents and chemicals

Not required

5. Procedure

Direct reading from the instrument

6. Alternative method

- Digital thermometer with a temperature probe (Fresenius, 1988)

NB: * A thermosphere type thermometer with appropriate cable length should be used to determine thermal stratification in deep waterbodies.

Working Range 0 - 40 NTU	Detection limit 0.02 NTU
Precision at 26 NTU 2% at 41 NTU 2%	
Interferences Presence of floating debris and coarse sediment which can settle down rapidly will give low readings Finely divided air bubbles will affect the results The presence of true colour (i.e. the colour of water arising out of the dissolved substances which can absorb light) will cause turbidities to be low	

1. **Application**

1.1 The method is applicable to potable, fresh and saline waters.

2. **Summary of method**

2.1 The method is based upon a comparison of the intensity of light scattered by the sample with the intensity of the light scattered by a standard reference suspension.

2.2 The higher the intensity of the light scattered, the higher the turbidity.

3. **Sampling**

3.1 Direct, unfiltered

4. **Preservation**

4.1 Store samples in the dark at 4 °C up to 48 hours.

5. **Apparatus**

5.1 A nephelometer with a light source and a photoelectric detector

5.2 Clear, colorless glass sample tubes

6. Reagents and chemicals

- 6.1 Turbidity free water
- 6.2 Hydrazine sulphate [(NH₂)₂.H₂SO₄]
- 6.3 Hexamethylene-tetraamine [(CH₂)₆N₄]

7 Preparation of standards and reagents

- 7.1 Turbidity free water
 - 7.1.1 Pass distilled water through a 0.45 μm pore size membrane filter paper
- 7.2 Stock formazin turbidity suspension.
 - 7.2.1 Solution I- Dissolve 1.00 g hydrazine sulphate in distilled water.
 - 7.2.2 Dilute to 100 ml in a volumetric flask.
 - 7.2.3 Solution II - Dissolve 10.00 g hexamethylene - tetramine in distilled water.
 - 7.2.4 Dilute to 100 ml in a volumetric flask.
 - 7.2.5 Mix 5.0 ml solution I with 5.0 ml solution II in a 100 ml volumetric flask.
 - 7.2.6 Allow to stand 24 hours at 25 ± 3 °C, then dilute to the mark and mix (this is the stock formazin turbidity suspension).
- 7.3 Standard formazin turbidity suspension
 - 7.3.1 Dilute 10.00 ml stock turbidity suspension to 100 ml with turbidity free water
 - 7.3.2 Dilute portions of the standard turbidity suspension with turbidity free water, as required.

8. Calibration

- 8.1 Follow the manufacturer's operating instructions.
- 8.2 Calibrate the instrument by using appropriate calibration standards prepared in 7.3.
- 8.3 Run at least one standard in the range to be used, each time prior to sample analysis.

9 Procedure

- 9.1 Turbidity less than 40 NTU units
 - 9.1.1 Shake the sample thoroughly to disperse the solids.
 - 9.1.2 When air bubbles disappeared, pour the sample into the nephelometer tube.
 - 9.1.3 Read the turbidity directly from the instrument.

- 9.2 Turbidity exceeding 40 NTU units
 - 9.2.1 Dilute the sample with one or more equal volumes of turbidity free water until the turbidity falls below 40 NTU units.
 - 9.2.2 Shake the diluted sample thoroughly and when the air bubbles disappear, pour the sample into the nephelometer tube.
 - 9.2.3 Read the turbidity directly from the instrument.
 - 9.2.4 Compare the turbidity of the original sample from the turbidity of the diluted sample and the dilution factor.

10. **Calculation**

- 10.1 Multiply sample reading by appropriate dilution to obtain the final reading.

$$\text{Turbidity, NTU} = \frac{A \times (V_1 + V_2)}{V_2}$$

- where:
- A = NTU found in diluted sample
 - V₁ = volume of dilution water (ml)
 - V₂ = original sample volume taken for dilution (ml)

11. **Alternative method**

- Visual method (Suess, 1982)

TOTAL HARDNESS mg l^{-1} as CaCO_3

Titrimetric

Working Range: All concentration ranges of hardness (but preferably below 25 mg as CaCO_3 in the sample aliquot)

Precision	For synthetic water samples:	Accuracy	at 31 mg l^{-1} CaCO_3 , -0.87%
	at 31 mg l^{-1} CaCO_3 , 9%		at 444 mg l^{-1} CaCO_3 , -3.23%
	at 444 mg l^{-1} CaCO_3 , 2%		

Interferences: Excessive amounts of heavy metals

1. Application

1.1 The method is applicable to potable, fresh and saline waters and effluents.

2. Summary of method

2.1 Calcium and magnesium ions in the sample are titrated upon the addition of disodium ethylenediamine tetraacetate (EDTA sodium form).

2.2 The end point is detected by means of Eriochrome Black-T indicator

3. Sampling

3.1 Direct, filtered

4. Preservation

4.1 Store at 4 °C

5. Apparatus

5.1 Standard laboratory titrimetric equipment

6. Reagents and chemicals

6.1 Magnesium sulphate [$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$]

6.2 Ammonium chloride [NH_4Cl]

6.3 Conc. ammonium hydroxide [NH_4OH]

6.4 Sodium cyanide [NaCN]

- 6.5 Sodium sulfide [$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$]
- 6.6 Calcium carbonate [CaCO_3]
- 6.7 Conc. hydrochloric acid [HCl]
- 6.8 Methyl red
- 6.9 Disodium ethylenediamine tetraacetate [Na_2EDTA]
- 6.10 Hydroxylamine hydrochloride [$\text{NH}_2\text{OH} \cdot \text{HCl}$]
- 6.11 Ethanol or isopropanol (95%) [$\text{C}_2\text{H}_5\text{OH}$ or $\text{C}_3\text{H}_7\text{OH}$]
- 6.12 Erichrome Black-T

7. Preparation of standards and reagents

- 7.1 Buffer solution (MgEDTA solution)
 - 7.1.1 Dissolve 1.179 g disodium EDTA and 780 mg magnesium sulphate in 50 ml distilled water. Add this solution to a 250 ml volumetric flask containing 16.9 g ammonium chloride and 143 ml conc. ammonium hydroxide with mixing and dilute to the mark with distilled water.
 - 7.1.2 Store this solution in a tightly stoppered plastic bottle (discard this solution when 1 or 2 ml added to the sample fails to produce a pH of 10.0 ± 0.1 at the end point of titration).
- 7.2 Inhibitors
 - 7.2.1 Inhibitor I - NaCN powder
 - 7.2.2 Inhibitor II - Dissolve 5.0 g sodium sulfide ($\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$) or 3.7 g sodium sulfide ($\text{Na}_2\text{S} \cdot 5\text{H}_2\text{O}$) in 100 ml distilled water. Exclude air with tightly fitted rubber stopper.
 - 7.2.3 Inhibitor III - Dissolve 4.5 g hydroxylamine hydrochloride in 100 ml of 95% ethanol or isopropanol.
- 7.3 Erichrome Black-T indicator solution
 - 7.3.1 Mix 0.5 g of Erichrome Black-T with 4.5 g of hydroxylamine hydrochloride.
 - 7.3.2 Dissolve in 100 ml of 95% ethanol or isopropanol.
- 7.4 HCl (1+1)
 - 7.4.1 Add 1 volume of conc. HCl to 1 volume of deionized distilled water.
- 7.5 Ammonium hydroxide solution (1 N)
 - 7.5.1 Dilute 70 ml of conc. NH_4OH to 1 liter with distilled water.
- 7.6 Standard CaCO_3 solution (0.02 N)
 - 7.6.1 Place 1.00 g of anhydrous CaCO_3 in a 500 ml beaker.
 - 7.6.2 Add HCl (1+1) gradually until all the CaCO_3 is dissolved.
 - 7.6.3 Add 200 ml distilled water.
 - 7.6.4 Boil for few minutes and cool.
 - 7.6.5 Add few drops of methyl red indicator and adjust to

intermediate orange colour by adding 1 N NH₄OH solution or (1+1) HCl as required.

7.6.6 Quantitatively transfer to a 1 liter volumetric flask and dilute to the mark with distilled water.

7.7 Standard EDTA titrant (approximate 0.02 N)

7.7.1 Place 3.723 g analytical reagent grade disodium EDTA in one litre volumetric flask.

7.7.2 Dilute to the mark with distilled water.

7.7.3 Standardize the EDTA solution with standard calcium solution (7.6) by titration procedure given in 8.1.

7.7.4 Label and store in a polyethylene bottle.

8. Procedure

8.1 Standardization of EDTA titrant

8.1.1 Place 10.0 ml of standard calcium solution (7.6) in a conical flask containing 50 ml of distilled water.

8.1.2 Add 1 ml buffer solution.

8.1.3 Add 1 to 2 drops of the Erichrome Black T indicator.

8.1.4 Titrate slowly with EDTA solution (7.7) until the last reddish colour disappears (red ⇒ blue).

8.2 Place 25.0 ml sample in titration flask, neutralize with 1 N ammonium hydroxide or with HCl (1+1).

8.3 Dilute to about 50 ml.

8.4 Add 1 or 2 ml of the buffer solution (see *NB*).

8.5 If the end point is not sharp (as determined by practice run), add inhibitors at this point.

8.6 Add 1 or 2 drops of indicator solution.

8.7 Titrate slowly while stirring with standard EDTA titrant (7.7) until the last reddish tint disappears. Solution is normally blue at end point (red ⇒ blue).

9. Calculation

$$\text{Normality of EDTA} = \frac{0.2}{\text{volume of EDTA (ml)}}$$

$$\text{Hardness (EDTA) } \text{mg l}^{-1} \text{ as CaCO}_3 = \frac{V \times A \times 1000}{\text{sample volume (ml)}}$$

where,

V	=	volume of EDTA titrant used for sample in ml
A	=	mg CaCO ₃ equivalent to 1.00 ml EDTA titrant

10. **Alternative method**

- Direct calculation after determination of calcium and magnesium by AAS (USEPA, 1983).

- NB:*
- * For low hardness (less than 5 mg l⁻¹) , a large sample and proportionately larger amounts of buffer, inhibitor and indicator must be used.
 - * Inhibitors are used to reduce the metallic interferences.
 - * To avoid large titration volumes, use a sample aliquot containing not more than 25 mg l⁻¹ CaCO₃.
 - * Refer AAS method for Ca and Mg determination if alternative method is used for hardness.

Precision	± 0.02 pH units	Accuracy	± 0.05 pH units ± 0.1 pH units (limit of accuracy under normal conditions)
Interferences	Dissolved gases such as CO ₂ , H ₂ S or NH ₃ may contribute to acidity Solids and oily matter may coat the glass electrode surface Presence of oxidizable or hydrolyzable ions may interfere. Na may interfere at pH levels greater than 10 (alkaline error) Measurements are not reliable below pH 1 (acid error).		

1. Application

- 1.1 pH can be operationally defined as the H⁺ concentration in log scale .
- 1.2 This method is applicable to potable, fresh and saline waters and effluents.

2. Summary of method

- 2.1 The pH is determined electrometrically using either a combination type pH (glass) electrode or glass electrode coupled with a standard reference electrode.

3. Sampling

- 3.1 *In situ* measurements

4. Apparatus

- 4.1 pH meter (laboratory or field model)
- 4.2 Combination pH electrode (or a glass electrode coupled with a standard reference electrode)
- 4.3 Magnetic stirrer and teflon coated stirring bar
- 4.4 Thermometer

5. Reagents and chemicals

- 5.1 Buffer standards: pH = 4.0, pH = 7.0 and pH = 9.0 (see NB)

6. Preparation of standards and reagents

- 6.1 Buffer standard solutions may be purchased directly from chemical manufacturers or prepared according to the procedures given in the *NB*.

7. Calibration

- 7.1 Each instrument/electrode system must be calibrated with minimum of 2 buffer solutions having pH values approximately three pH units apart from the expected pH of the sample.

8. Procedure

- 8.1 Keep electrodes wet by returning them to storage solution (standard KCl solution) whenever pH meter is not in use.
- 8.2 Standardize the meter and electrode system using pH buffer solutions as outlined in Chapter 1.5.
- 8.3 Place the sample solution in a clean glass beaker (a sufficient volume to cover the sensing elements of the electrodes should be taken).
- 8.4 After rinsing and gently wiping the electrodes, (or combination electrode) immerse them into the sample and stir at a constant rate to provide homogeneity.
- 8.5 Record sample pH and temperature.
- 8.6 Repeat measurement on successive portions of the sample until values differ by less than 0.05 pH units. Two or three volume changes are usually sufficient (if the sample temperature differs by more than 2 °C from the buffer solution the measured pH values must be corrected).

9. Calculation

- 9.1 pH meters display readings directly in pH units.
- 9.2 Report pH to the nearest 0.05 pH unit and temperature to the nearest degree in Celsius.

10. Alternative method

- Colorimetric method (Suess, 1982)

- NB:*
- * Preparation of buffer standard, pH = 4.004 at 25 °C
Dissolve 10.12 g of potassium hydrogen phthalate [$\text{KHC}_8\text{H}_4\text{O}_4$] in distilled water and dilute to 1000 ml.
 - * Preparation of buffer standard, pH = 9.183 at 25 °C

Dissolve 3.80 g of sodium borate decahydrate [$\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$] (borax) in distilled water and dilute to 1000 ml.

* Preparation of buffer standard, $\text{pH} = 7.415$ at 25°C

Dissolve 1.179 g of potassium dihydrogen phosphate [KH_2PO_4] and 4.303 g of disodium hydrogen phosphate [Na_2HPO_4] in 1000 ml distilled water.

* For electrode cleaning refer Chapter 2.3

Working Range: 1 - 10,000 $\mu\text{mhos cm}^{-1}$ (μS)

Precision. at 100 $\mu\text{mhos cm}^{-1}$ 8 %
at 1710 $\mu\text{mhos cm}^{-1}$ 7 %

Accuracy at 100 $\mu\text{mhos cm}^{-1}$ -2.02 %
at 1710 $\mu\text{mhos cm}^{-1}$ -5.08 %

Interferences: No significant interferences

1. Application

Method is applicable to potable, fresh and saline waters and effluents.

2. Summary of method

2.1 The specific conductance of a sample is measured by use of a self-contained conductivity meter, Wheatstone bridge type or equivalent.

3. Sampling

3.1 *In situ* measurements

4. Apparatus

4.1 Conductivity bridge, range 1 to 10,000 $\mu\text{mhos cm}^{-1}$ with a cell constant of 1.0

4.2 Thermometer

5. Reagents and chemicals

5.1 Conductivity water

5.2 Potassium chloride [KCl]

6. Preparation of standards and reagents

6.1 Conductivity water

6.1.1 Pass distilled water through a mixed-bed deionizer and discard water till the conductivity is less than 1 $\mu\text{mhos cm}^{-1}$ (usually this condition is attained within 1 litre of water).

6.2 Standard KCl solution (0.01 M)

6.2.1 Dissolve 0.7456 g of pre-dried (2 hours at 105 °C) KCl in

conductivity water and dilute to 1 liter at 25 °C.

7. Calibration

- 7.1 Instrument must be calibrated with KCl (0.01 M) solution before use, by adjusting the meter reading to the conductivity of 0.01 M KCl solution at the measurement temperature (i.e. sample temperature) as given below.

For 0.01 M KCl solution

°C	Conductivity $\mu\text{mhos cm}^{-1}$
21	1305
22	1332
23	1359
24	1386
25	1413
26	1441
27	1468
28	1496

8. Procedure

- 8.1 Rinse the cell with distilled water followed by one portion of the sample water
- 8.2 Measure the sample conductivity and note the water temperature.
- 8.3 If conversion of sample conductivity to 25 °C is desired,
- (a) Add 2% of the sample reading per degree if the temperature of the sample is below 25 °C.
 - (b) Subtract 2% of the sample reading per degree if the temperature of the sample is above 25 °C.

NB: * Temperature variations and corrections represent the largest source of potential error.

Working Range: 4 - 20,000 mg l⁻¹

Interferences: Samples with high concentration of dissolved solids

1. Application

1.1 The method is applicable to potable, fresh and saline waters and effluents.

2. Summary of method

2.1 A known volume of well stirred sample is filtered through a preweighed (dried at 105 °C) glass fiber filter and the residue retained on the filter is dried to constant weight at 103-105 °C.

3. Sampling

3.1 Direct unfiltered

4. Preservation

4.1 If preservation of samples is not practicable, analysis should begin as soon as possible. Till then store at 4 °C.

5. Apparatus

5.1 Glass fiber filter discs without organic binder (0.45 μm pore size, 4.7 cm diameter)

5.2 Filtering apparatus with reservoir and a coarse (40 -60 microns) fritted disc as a filter support

5.3 Suction flask

5.4 Drying oven

5.5 Desiccator

5.6 Analytical balance capable of weighing to 0.1 mg

6. Procedure

- 6.1 Preparation of glass fiber filter
 - 6.1.1 Place the glass fiber filter on the membrane filter apparatus or insert into the bottom of a suitable Gooch crucible with the wrinkled surface facing upwards.
 - 6.1.2 While vacuum is applied, wash the disc with three successive 20 ml volumes of distilled water.
 - 6.1.3 After water has passed through, remove all traces of water by continuous application of vacuum.
 - 6.1.4 Remove the filter from membrane filter apparatus, or, both crucible and filter if Gooch crucible is used, and dry in an oven at 103-105 °C for one hour.
 - 6.1.5 Remove the desiccator, cool and weigh.
 - 6.1.6 Repeat the drying cycle until a constant weight is obtained
- 6.2 Selection of sample volume
 - 6.2.1 For a 4.7 cm diameter (0.45 μm pore size) filter, take 100 ml of sample for filtration.
 - 6.2.2 If weight of retained residue is less than 1.0 mg, the sample volume must be increased to provide at least 1.0 mg of residue.
 - 6.2.3 If other filter diameters are used, start with a sample volume equal to 7 mlcm⁻² of filter area and collect at least a weight of residue equivalent to 1.0 mg.
- 6.3 Assemble the filtering apparatus, place the prepared glass fiber filter on the filter support and begin suction (wet the filter with a small amount of distilled water first).
- 6.4 Shake the sample vigorously and take predetermined sample volume into a graduated cylinder and then transfer it quantitatively to the filter.
- 6.5 Remove all traces of water by continuous application of vacuum.
- 6.6 With suction on, wash the graduated cylinder, the filter containing non filterable residue and the filter funnel wall with three volume portions of distilled water allowing complete drainage between washings.
- 6.7 Remove the filter from the filter support (remove crucible and filter from crucible adapter if Gooch crucible is used).
- 6.8 Dry at least for twelve hours at 103-105 °C in an oven.
- 6.9 Cool in a desiccator and weigh.
- 6.10 Repeat 6.8-6.9 till a constant weight (difference within ± 0.5 mg) is obtained.

7. Calculation

$$\text{Non filterable residue, mg l}^{-1} = \frac{(A-B) \times 1000}{V}$$

where;

A	=	weight of filter (or filter + crucible) + residue in mg
B	=	weight of filter (or filter + crucible) in mg
V	=	volume of sample filtered in ml

- NB:*
- * Non representative particulates such as leaves, sticks, fish and lumps of faecal matter should be excluded from the sample if their inclusion is not desired in the final result.
 - * Care must be taken in selecting the filtering apparatus for waters containing high dissolved solid (brine, waste water) in order to avoid retention of dissolved solids.

Working Range: 10 - 20,000 mg^l⁻¹

Interferences. High concentrations of dissolved calcium, magnesium, chloride, sulphate and bicarbonate will interfere
Excessive residue in the dish may form a crust which entraps water that will not be driven off during drying

1. **Application**

1.1 The method is applicable to potable, fresh and saline waters and effluents.

2. **Summary of method**

2.1 A known volume of well stirred sample is filtered through a standard glass fiber filter. The filtrate is evaporated and dried to constant weight at 180 °C.

3. **Sampling**

3.1 Direct filtered through 0.45 μm membrane filter

4. **Preservation**

4.1 Preserve at 4 °C.

5. **Apparatus**

- 5.1 Glass fiber filter discs without organic binder (0.45 μm pore size, 4.7 cm or 2.1 cm diameter)
- 5.2 Filter holder, membrane filter funnel or Gooch crucible adapter
- 5.3 Suction flask, 500 ml
- 5.4 Gooch crucible, 25 ml (if 2.1 cm filter is used).
- 5.5 Evaporating dishes (porcelain, 100 ml volume)
- 5.6 Steam bath
- 5.7 Drying oven
- 5.8 Desiccator
- 5.9 Analytical balance capable of weighing to 0.1 mg

6. Procedure

- 6.1 Preparation of glass fiber filter
 - 6.1.1 Place the glass fiber filter on the membrane filter apparatus or insert the filter into the bottom of a suitable Gooch crucible.
 - 6.1.2 While vacuum is applied wash the disc with three successive 20 ml volumes of distilled water.
 - 6.1.3 After water has passed through, remove all traces of water by continuous application of vacuum. Discard washings.
- 6.2 Preparation of evaporating dishes
 - 6.2.1 Heat the clean dish to 180 ± 2 °C for one hour in an oven.
 - 6.2.2 Cool in a desiccator, weigh and store until needed.
- 6.3 Assemble the filtering apparatus, place the prepared filter on the filter support and begin suction (wet the filter with a small amount of distilled water first).
- 6.4 Shake the sample vigorously, transfer 125 ml to the funnel by means of a 100 ml graduated cylinder.
- 6.5 Filter the sample through the glass fiber filter.
- 6.6 Rinse the graduated cylinder, the filter and the funnel wall with three 10 ml portions of distilled water and continue to apply vacuum for about 3 minutes.
- 6.7 Transfer 100 ml of the filtrate to a pre-weighed evaporating dish.
- 6.8 Evaporate to dryness on a steam bath.
- 6.9 Dry the evaporated sample at least for one hour at 180 ± 2 °C in an oven.
- 6.10 Cool in a desiccator and weigh. Repeat the drying/weighing cycle until a constant weight is obtained or until weight loss is less than 0.5 mg.

7. Calculation

$$\text{Filterable residue, } \text{mg l}^{-1} = \frac{(A-B) \times 1000}{V}$$

where; A = weight of the dried residue + dish in mg
 B = weight of the empty dish in mg
 V = volume of sample used in ml

8. Alternative method

- Direct calculation after determining respective cations and anions (APHA, 1989).

- NB:*
- * Highly mineralized and samples containing high concentrations of bicarbonate will require prolonged drying at 180 °C.
 - * Total residue in the evaporating dish should be limited to about 200 mg.

5.3 Metals

ALUMINUM $\mu\text{g l}^{-1}$

Chelation/Extraction - AAS

Lamp: aluminum hollow cathode

Wavelength 309.3 nm

Fuel acetylene

Oxidant nitrous oxide

Working Range 10 - 300 $\mu\text{g l}^{-1}$ **Precision** at 50 $\mu\text{g l}^{-1}$ $\pm 12\%$
at 300 $\mu\text{g l}^{-1}$ $\pm 10\%$ **Accuracy** at 40 $\mu\text{g l}^{-1}$ +5.8%
at 275 $\mu\text{g l}^{-1}$ -3.5%**Interferences** Iron concentrations above 10 mg l^{-1}
Magnesium forms an insoluble quinolate at pH 8

1. Application

1.1 The method is applicable to potable, fresh and saline waters and effluents.

2. Summary of method

- 2.1 Aluminum is extracted into methylisobutyl ketone (MIBK), after chelation with 8-hydroxyquinoline.
- 2.2 The extract is aspirated directly into AAS with a nitrous oxide-acetylene flame.

3. Sampling

3.1 Direct, filtered through 0.45 μm membrane filter paper.

4. Preservation

4.1 Acidified with nitric acid to $\text{pH} < 1$

5. Apparatus

5.1 Atomic Absorption Spectrophotometer

6. Reagents, chemicals and gases

6.1 Aluminum potassium sulphate $[\text{AlK}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}]$

- 6.2 Conc. ammonium hydroxide [NH₄OH]
- 6.3 Ammonium acetate [NH₄C₂H₃O₂]
- 6.4 8-hydroxyquinoline
- 6.5 Methyl isobutyl ketone (MIBK) [C₆H₁₂O]
- 6.6 Glacial acetic acid [C₂H₄O₂]
- 6.7 Conc. nitric acid (ultra pure) [HNO₃]
- 6.8 Conc. hydrochloric acid [HCl]
- 6.9 Nitrous oxide [N₂O]
- 6.10 Compressed air
- 6.11 Acetylene

7. Preparation of standards and reagents

- 7.1 Standard Aluminum solution (1 ml ≡ 100 μg Al). Obtain commercially available AAS standard or prepare manually as follows:
 - 7.1.1 Dissolve 1.758 g of aluminum potassium sulphate in distilled water
 - 7.1.2 Add 1 ml of conc. nitric acid.
 - 7.1.3 Dilute to 1 liter with distilled water.
- 7.2 Working Aluminum solution (1 ml ≡ 1.0 μg Al)
 - 7.2.1 Dilute 10 ml of the standard aluminum solution, adding 1 ml of nitric acid, to 1 liter with distilled water.
- 7.3 Ammonium hydroxide-ammonium acetate buffer
 - 7.3.1 Dissolve 200 g of ammonium acetate in distilled water.
 - 7.3.2 Add 70 ml of concentrated ammonium hydroxide.
 - 7.3.3 Dilute to 1 liter with distilled water.
- 7.4 8-hydroxyquinoline solution
 - 7.4.1 Dissolve 20 g of 8-hydroxyquinoline in a mixture of 57 ml of glacial acetic acid and 200 ml of distilled water.
 - 7.4.2 Dilute to 1 liter with distilled water.
- 7.5 MIBK saturated water/ water saturated MIBK
 - 7.5.1 In a separatory funnel mix equal volumes of MIBK and water and separate phases carefully.
 - 7.5.2 Use **MIBK phase** (water saturated MIBK) in 9.9 and 9.12.
 - 7.5.3 Use **water phase** (MIBK saturated water) in 9.11.

8 Calibration

- 8.1 Prepare a blank and standard ranging in concentration from 10 μg l⁻¹ to 300 μg l⁻¹ by appropriate dilution of the working aluminum solution (7.2).

- 8.2 Extract 100 ml of each standard and a blank following the procedure 9.1-9.12.
- 8.3 Switch on the AAS and optimize instrumental parameters (refer Chapter 1.7.4).
- 8.4 Aspirate the sample blank to AAS.
- 8.5 Set zero absorbance.
- 8.6 Aspirate standards and record the absorbance.
- 8.7 Plot the calibration curve, absorbance versus concentration of aluminum of the standards (in modern AAS, calibration curve is automatically plotted in the data processing unit of the instrument).

9. Procedure

- 9.1 Soak all glassware in hot HCl (1+1) for 2 hours. Drain and rinse at least 5 times with water. Drain and flush with methyl, ethyl or isopropyl alcohol.
- 9.2 Measure a well mixed acidified sample volume upto a maximum of 100 ml into a 125 ml beaker.
- 9.3 Add 0.5 ml of nitric acid and 5 ml of hydrochloric acid.
- 9.4 Heat the sample on a hot plate until the volume has been reduced to 10 to 15 ml (do not boil the sample).
- 9.5 Adjust the pH to 8 with concentrated ammonium hydroxide.
- 9.6 If necessary, filter the sample into a 200 ml volumetric flask and wash the filter paper several times with distilled water.
- 9.7 Bring the volume approximately to 100 ml in a 200 ml flask.
- 9.8 Add 2 ml of 8-hydroxyquinoline solution and mix.
- 9.9 Add 10 ml of buffer solution to the flask and immediately add 10 ml of **MIBK phase**.
- 9.10 Shake vigorously for 15 seconds.
- 9.11 After the layers have separated add **water phase** to raise the ketone layer completely upto the neck of the flask.
- 9.12 Aspirate the ketone layer into the nitrous oxide-acetylene flame and measure its absorbance (aspirate **MIBK phase** between samples).
- 9.13 Read the aluminum concentration in the **MIBK phase** from the calibration curve.

10. Calculation

$$\text{Aluminum, } \mu\text{g l}^{-1} = C \times \frac{100}{V}$$

where; C = concentration from the curve in $\mu\text{g l}^{-1}$
 V = sample volume as measured in 9.2 in ml

11. **Alternative method**

- Spectrophotometry with catecholate violet method (Suess, 1982)

NB: * Interference caused by magnesium can be avoided if the samples are extracted immediately after they are buffered to pH 8.

CADMIUM $\mu\text{g l}^{-1}$

Chelation/Extraction - AAS
Lamp cadmium hollow cathode
Wavelength 228.8 nm
Fuel: acetylene
Oxidant air

Working Range 5 - 200 $\mu\text{g l}^{-1}$

Precision at 30 $\mu\text{g l}^{-1}$ 23%
at 160 $\mu\text{g l}^{-1}$ 13%

Accuracy at 77 $\mu\text{g l}^{-1}$ -3.6%
at 29 $\mu\text{g l}^{-1}$ -3.1%

Interferences Ca > 1000 mg l^{-1} , Pb > 6 mg l^{-1} ; Zn > 3 mg l^{-1} ; Cu > 1 mg l^{-1}

1. Application

1.1 Method is applicable to potable, fresh and saline waters and effluents

2. Summary of method

- 2.1 Cadmium is chelated with ammonium pyrrolidine dithiocarbamate (APDC) and extracted into chloroform.
- 2.2 The extract is evaporated and treated with nitric acid to destroy organic matter following which the residue is dissolved in hydrochloric acid.
- 2.3 The cadmium in the resulting solution is then determined by aspirating to AAS.

3. Sampling

3.1 Direct, filtered through 0.45 μm membrane filter paper.

4. Preservation

4.1 Acidified with nitric acid to $\text{pH} < 1$

5. Apparatus

5.1 Atomic Absorption Spectrophotometer

6. Reagents, chemicals and gases

- 6.1 Bromphenol blue indicator solution
- 6.2 AAS standard cadmium solution or cadmium metal

- 6.3 Chloroform [CHCl_3]
- 6.4 Ethanol or Isopropanol [$\text{C}_2\text{H}_5\text{OH}$ or $\text{C}_3\text{H}_7\text{OH}$]
- 6.5 Carbon disulfide [CS_2]
- 6.6 Ammonium pyrrolidine dithiocarbamate (APDC) [$\text{C}_5\text{H}_8\text{NS}_2\cdot\text{NH}_4$]
- 6.7 Conc. hydrochloric acid [HCl]
- 6.8 Conc. nitric acid (ultra pure) [HNO_3]
- 6.9 Sodium hydroxide [NaOH]
- 6.10 Compressed air
- 6.11 Acetylene

7. Preparation of standards and reagents

- 7.1 Bromphenol blue indicator solution (1 g l^{-1})
 - 7.1.1 Dissolve 0.1 g of bromphenol blue in 100 ml of 50% ethanol or isopropanol.
- 7.2 Standard Cadmium stock solution ($1 \text{ ml} \equiv 1 \text{ mg Cd}$). Obtain commercially available AAS standard or prepare manually as follows:
 - 7.2.1 Dissolve 1.000 g of cadmium metal in a minimum quantity of nitric acid.
 - 7.2.2 Dilute to 1 liter with distilled water.
- 7.3 Intermediate Cadmium solution ($1 \text{ ml} \equiv 50 \mu\text{g Cd}$)
 - 7.3.1 Dilute 50 ml of the stock solution (7.2) and 1 ml of nitric acid to 1 liter with distilled water (prepare weekly).
- 7.4 Working standard Cadmium solution ($1 \text{ ml} \equiv 0.5 \mu\text{g Cd}$)
 - 7.4.1 Dilute 10 ml of Cd intermediate solution (7.3) and 1 ml of nitric acid to 1 liter with distilled water (prepare daily).
- 7.5 Hydrochloric acid (1+2)
 - 7.5.1 Add 1 volume of HCl to 2 volumes of water.
- 7.6 Hydrochloric acid (1+49)
 - 7.6.1 Add 1 volume of HCl to 49 volumes of water.
- 7.7 APDC/ chloroform reagent
 - 7.7.1 Add 9 ml of APDC to 250 ml of chloroform.
 - 7.7.2 Cool the solution and add 7.5 ml of carbon disulfide in small portions.
 - 7.7.3 Dilute to 500 ml with chloroform.
- 7.8 Sodium hydroxide solution (100 g l^{-1})
 - 7.8.1 Dissolve 100 g of Sodium hydroxide in 1 l of distilled water.

8. Calibration

- 8.1 Prepare cadmium standards within the linear range ($5 - 200 \mu\text{g l}^{-1}$), using the working cadmium solution; make all the dilutions using

- distilled water.
- 8.2 Perform procedure 9.1 to 9.19 replacing sample with the sample blank (prepared in distilled water) and standards to preconcentrated cadmium. Use 100 ml of each standard and the blank.
 - 8.3 Switch on the AAS and optimize instrumental parameters (refer Chapter 1.7.4)
 - 8.4 Aspirate the preconcentrated sample blank.
 - 8.5 Set zero absorbance.
 - 8.6 Aspirate preconcentrate of standards and record the absorbance.
 - 8.7 Plot the calibration curve, absorbance versus concentration of cadmium standards.

9. Procedure

- 9.1 Measure a volume of (100 ml maximum) well mixed sample into a 125 ml beaker.
- 9.2 Adjust the volume to 100 ml with distilled water, if necessary.
- 9.3 Add 5 ml of conc. HCl.
- 9.4 Heat the sample in a steam bath or on a hot plate until the volume is reduced to 15 to 20 ml (do not boil the sample).
- 9.5 Cool and filter the sample into a 250 ml separatory funnel.
- 9.6 Wash the filter paper with distilled water into the funnel and adjust the volume approximately to 100 ml.
- 9.7 Add 2 drops of bromphenol blue indicator and mix well.
- 9.8 Adjust the pH by adding NaOH solution until a blue color persists.
- 9.9 Add HCl (1+49) dropwise until the blue color disappears.
- 9.10 Add 2.5 ml of HCl (1+49) in excess.
- 9.11 Add 10 ml of APDC/chloroform reagent and shake for few minutes.
- 9.12 Plug the tip of the separatory funnel with cotton wool, allow the phases to separate and drain the chloroform layer into a 100 ml beaker.
- 9.13 Repeat the extraction with 10 ml of chloroform and drain the chloroform layer into the same beaker.
- 9.14 Evaporate the chloroform layer to near dryness in a water bath under a well ventilated fume hood.
- 9.15 Remove beaker from heat and allow residual solvent to evaporate without further heating.
- 9.16 Add 2 ml nitric acid dropwise and mix well.
- 9.17 Evaporate the solution to near dryness.
- 9.18 Add 2 ml of HCl (1+2) to the beaker, and heat for 1 minute while swirling.
- 9.19 Cool and transfer the solution quantitatively to a 10 ml volumetric flask and adjust the volume with distilled water.

CALCIUM mg^l⁻¹

Atomic Absorption Spectrophotometric
Lamp calcium hollow cathode
Wavelength: 422.7 nm
Fuel acetylene
Oxidant: air
Flame. reducing

Working Range: 0.2 - 7 mg^l⁻¹

Detection limit: 0.003 mg^l⁻¹

Precision. at 9 mg^l⁻¹ 3%
at 36 mg^l⁻¹ 2%

Accuracy at 9 mg^l⁻¹ 99%
at 36 mg^l⁻¹ 99%

Interferences: Mg > 1000 mg^l⁻¹; Na/K > 500 mg^l⁻¹, NO₃ > 500 mg^l⁻¹

1. Application

1.1 The method can be used for potable, fresh and saline waters and effluents.

2. Summary of method

- 2.1 The sample is aspirated into the flame where the element is atomized and absorbs incoming radiation of the hollow cathode lamp.
2.2 Attenuation of radiation is measured as absorbance.

3. Sampling

3.1 Direct, filtered through 0.45 μm membrane filter paper.

4. Preservation

4.1 Preserve at 4 °C.

5. Apparatus

5.1 Atomic Absorption Spectrophotometer

6. Reagents, chemicals and gases

- 6.1 AAS standard calcium solution or calcium carbonate [CaCO₃] (analytical grade)
6.2 Lanthanum oxide [La₂O₃]

- 6.3 Conc. hydrochloric acid [HCl]
- 6.4 Compressed air
- 6.5 Acetylene

7. Preparation of standards and reagents

- 7.1 Standard calcium stock solution (500 ppm Ca^{2+}). Obtain commercially available AAS standard or prepare manually as follows:
 - 7.1.1 Dissolve 1.250 g of calcium carbonate (dried at 180 °C for one hour before weighing) with a small quantity of deionized water.
 - 7.1.2 Dilute to 1000 ml with deionized distilled water.
- 7.2 Working standard calcium solution (10 ppm Ca^{2+})
 - 7.2.1 Prepare a 50 fold dilution of the stock solution with deionized distilled water.
- 7.3 Lanthanum chloride solution
 - 7.3.1 Dissolve 29 g of lanthanum oxide slowly in 250 ml conc. HCl by adding small portions at a time.
 - 7.3.2 Dilute to 500 ml with deionized distilled water.

8. Calibration

- 8.1 Prepare calcium standards within the linear range (0.2 - 7 mg l^{-1}), using working calcium solution (make all the dilutions with deionized distilled water).
- 8.2 To each 10 ml standard add 1 ml of lanthanum chloride solution and mix well.
- 8.3 Switch on the AAS and optimize instrumental parameters (refer Chapter 1.7.4).
- 8.4 Aspirate the sample blank (i.e. distilled water with 1 ml of lanthanum chloride).
- 8.5 Set zero absorbance.
- 8.6 Aspirate standards and record the absorbance.
- 8.7 Plot the calibration curve.

9. Procedure

- 9.1 Take 10 ml of the sample or an aliquot of the sample diluted to 10 ml.
- 9.2 Add 1.0 ml of the lanthanum chloride solution and mix well.
- 9.3 Aspirate the sample and record the absorbance.
- 9.4 Determine the concentration of calcium from the calibration curve.

10. **Calculation**

$$\text{Calcium, mg l}^{-1} = C \times D$$

where; C = concentration of Ca from the curve
 D = dilution factor

11. **Alternative method**

- EDTA titration (APHA, 1989)

- NB:*
- * Phosphate, sulphate and aluminum interferences can be eliminated by the addition of lanthanum ions.
 - * The nitrous oxide-acetylene flame will provide two to five times greater sensitivity and freedom from chemical interferences.
 - * The 239.9 nm line may also be used.

CHROMIUM $\mu\text{g l}^{-1}$

Spectrophotometric Wavelength 540 nm Path length 1 cm
--

Working Range. 10 - 500 $\mu\text{g l}^{-1}$

Precision : at 110 $\mu\text{g l}^{-1}$ 47.8%

Accuracy at 110 $\mu\text{g l}^{-1}$ 16.3%

Interferences: Mo > 200 mg l^{-1} , Hg > 200 mg l^{-1} ; V > 10 times of Cr;
Fe > 1 mg l^{-1}

1. Application

- 1.1 The method is applicable to potable, fresh and saline waters and effluents.
- 1.2 The procedure measures only hexavalent chromium, but other forms can also be determined after conversion to hexavalent form.

2. Summary of method

- 2.1 All the chromium is converted to the hexavalent state by oxidizing with potassium permanganate.
- 2.2 The hexavalent chromium is determined spectrophotometrically after complexation with diphenylcarbazide in acid solution.

3. Sampling

- 3.1 Direct, filtered through 0.45 μm membrane filter paper.

4. Preservation

- 4.1 Acidified with nitric acid to $\text{pH} < 1$

5. Apparatus

- 5.1 UV/Visible Spectrophotometer
- 5.2 Separating funnels (125 ml)

6. Reagents and chemicals

- 6.1 Potassium dichromate [$\text{K}_2\text{Cr}_2\text{O}_7$]

- 6.2 Conc nitric acid [HNO₃]
- 6.3 Conc. sulfuric acid [H₂SO₄]
- 6.4 Methyl orange
- 6.5 Hydrogen peroxide (30%)[H₂O₂]
- 6.6 Conc. ammonium hydroxide [NH₄OH]
- 6.7 Potassium permanganate [KMnO₄]
- 6.8 Sodium azide [NaN₃]
- 6.9 Diphenylcarbazide [C₁₃H₁₄N₄O]
- 6.10 Chloroform [CHCl₃]
- 6.11 Acetone [C₃H₆O]
- 6.12 Cupferron [C₆H₅N(NO)ONH₄]
- 6.13 Conc. phosphoric acid [H₃PO₄]
- 6.14 Sulfuric acid (6 N)[H₂SO₄]

7. Preparation of standards and reagents

- 7.1 Standard chromium stock solution. Obtain commercially available AAS standard or prepare manually as follows:
 - 7.1.1 Dissolve 141.4 mg potassium dichromate in distilled water and dilute to 1000 ml.
- 7.2 Working standard chromium solution
 - 7.2.1 Dilute 10.0 ml of the standard stock chromium solution to 100 ml.
- 7.3 Potassium permanganate solution
 - 7.3.1 Dissolve 4 g of potassium permanganate in 100 ml distilled water.
- 7.4 Sodium azide solution
 - 7.4.1 Dissolve 0.5 g of sodium azide in 100 ml distilled water
- 7.5 Diphenylcarbazide solution
 - 7.5.1 Dissolve 250 mg of 1,5-diphenylcarbazide in 50 ml acetone and **store in a brown bottle.**
- 7.6 Cupferron solution
 - 7.6.1 Dissolve 5 g of cupferron in 100 ml distilled water.
- 7.7 Sulfuric acid 0.2 N
 - 7.7.1 Dilute 17 ml 6 N H₂SO₄ to 500 ml with distilled water.
- 7.8 H₂SO₄ (1+1)
 - 7.8.1 Mix one volume of conc. H₂SO₄ with one volume of distilled water. (PRECAUTION: keep the diluting vessel in a water bath and add acid very slowly.)

8. Calibration

- 8.1 Measure appropriate aliquots of working standard chromium solution

(7.2), to give 10 to 100 μg Cr, into 250 ml beakers (volumes ranging from 2.0 to 20.0 ml).

- 8.2 Follow same pretreatment procedure as of the sample (9.1).
- 8.3 Set zero absorbance using the blank, prepared by the procedure replacing the sample with deionized distilled water.
- 8.4 Develop colour as for samples and measure absorbance at 540 nm.
- 8.5 Plot a calibration curve, absorbance versus chromium content.

9. Procedure

- 9.1 Pretreatment of sample
 - 9.1.1 If only hexavalent chromium is desired, no treatment is necessary. Directly proceed to 9.4.
 - 9.1.2 If total chromium is desired and there are molybdenum, vanadium, copper or iron present, proceed from 9.2 - 9.4.
 - 9.1.3 If the above metals are not present and total chromium is desired, follow the procedure described in 9.3 and 9.4.
- 9.2 Removal of molybdenum, vanadium, iron and copper with cupferron
 - 9.2.1 Pipette a portion of the original sample containing 10 to 100 μg Cr into a 125 ml separating funnel.
 - 9.2.2 Dilute to about 40 ml with distilled water and chill in an ice bath.
 - 9.2.3 Add 5 ml ice-cold cupferron solution, shake well and let it stand in ice bath for 1 minute.
 - 9.2.4 Extract in a separating funnel with three successive 5 ml portions of chloroform.
 - 9.2.5 Shake each portion thoroughly with aqueous solution, let layers separate, and discard chloroform extract.
 - 9.2.6 Transfer extracted aqueous solution to a 125 ml conical flask.
 - 9.2.7 Wash the funnel with a small amount of distilled water into the flask and boil for about 5 minutes and cool.
 - 9.2.8 Add 5 ml conc. HNO_3 and a minimum of 3 ml conc. H_2SO_4 acid.
 - 9.2.9 Boil samples until SO_3 fumes are observed.
 - 9.3.0 Cool and add 5 ml conc. HNO_3 , again boil to complete the decomposition of organic matter. Cool again and add 25 ml of distilled water
- 9.3 Oxidation of trivalent chromium
 - 9.3.1 Pipette a portion of the original sample containing 10 to 100 μg Cr (or the total sample subjected to 9.2) into a 125 ml conical flask.
 - 9.3.2 Using methyl orange as the indicator, add conc. NH_4OH until solution is just basic to methyl orange.
 - 9.3.3 Add H_2SO_4 (1+1) dropwise until it is acidic and add 1 ml in

COPPER $\mu\text{g l}^{-1}$

Chelation/Extraction - AAS Lamp copper hollow cathode Wavelength 324.7 nm Fuel acetylene Oxidant. air
--

Working Range 2 - 500 $\mu\text{g l}^{-1}$

Precision at 9 $\mu\text{g l}^{-1}$ 7%
at 36.3 $\mu\text{g l}^{-1}$ 25%

Accuracy: at 9 $\mu\text{g l}^{-1}$ +4%
at 36.3 $\mu\text{g l}^{-1}$ +4.3%

Interferences: Cd, Pb, Ni, Zn, Co, Mn, Cr > 10 mg l^{-1}

1. Application

1.1 The method is applicable to potable, fresh and saline waters and effluents.

2. Summary of method

2.1 Copper is chelated with ammonium pyrrolidine dithiocarbamate (APDC) and extracted into chloroform.

2.2 The extract is treated with nitric acid to destroy organic matter and is dissolved in hydrochloric acid.

2.3 The copper in the resulting solution is then determined by aspirating to AAS.

3. Sampling

3.1 Direct, filtered through 0.45 μm membrane filter paper

4. Preservation

4.1 Acidified with nitric acid to $\text{pH} < 1$

5. Apparatus

5.1 Atomic Absorption Spectrophotometer

6. Reagents, chemicals and gases

6.1 Bromphenol blue

6.2 AAS standard copper solution or pure copper metal

- 6.3 Chloroform [CHCl_3]
- 6.4 Ammonium pyrrolidine dithiocarbamate (APDC) [$\text{C}_5\text{H}_8\text{NS}_2\cdot\text{NH}_4$]
- 6.5 Ethanol or Isopropanol [$\text{C}_2\text{H}_5\text{OH}$ or $\text{C}_3\text{H}_7\text{OH}$]
- 6.6 Conc. hydrochloric acid [HCl]
- 6.7 Conc. nitric acid (ultra pure) [HNO_3]
- 6.8 Sulfuric acid (1+1) [H_2SO_4]
- 6.9 Sodium hydroxide [NaOH]
- 6.10 Compressed air
- 6.11 Acetylene

7. Preparation of standards and reagents

- 7.1 Bromphenol blue indicator solution (1 g l^{-1})
 - 7.1.1 Dissolve 0.1 g of bromphenol blue in 100 ml of 50% ethanol or isopropanol.
- 7.2 Standard copper stock solution ($1 \text{ ml} \equiv 1 \text{ mg Cu}$). Obtain commercially available AAS standard or prepare manually as follows:
 - 7.2.1 Dissolve 1 g of electrolytic copper in a mixture of 15 ml nitric acid and 15 ml deionized distilled water in a 250 ml beaker.
 - 7.2.2 Slowly add 4 ml of sulfuric acid (1+1) and heat until SO_3 fumes evolve.
 - 7.2.3 Cool, wash down the walls of the beaker with deionized distilled water and dilute to 1 liter in a volumetric flask.
- 7.3 Intermediate copper solution ($1 \text{ ml} \equiv 10 \mu\text{g Cu}$)
 - 7.3.1 Dilute 10 ml of the stock solution (7.2) and 1 ml of nitric acid to 1 liter with deionized distilled water.
- 7.4 Working standard copper solution ($1 \text{ ml} \equiv 1 \mu\text{g Cu}$)
 - 7.4.1 Dilute 10 ml of the intermediate copper solution (7.3) to 100 ml with deionized distilled water
- 7.5 Hydrochloric acid (1+2)
 - 7.5.1 Add 1 volume of HCl to 2 volumes of deionized distilled water.
- 7.6 Hydrochloric acid (1+49)
 - 7.6.1 Add 1 volume of HCl to 49 volumes of deionized distilled water
- 7.7 APDC/chloroform reagent
 - 7.7.1 Add 36 ml of APDC to 1 liter of chloroform.
 - 7.7.2 Cool the solution and add 30 ml of carbon disulfide in small portions.
 - 7.7.3 Dilute to 2 liters with chloroform.
- 7.8 Sodium hydroxide solution (100 g l^{-1})
 - 7.8.1 Dissolve 100 g of sodium hydroxide in distilled water and dilute to 1 liter.

8. Calibration

- 8.1 Prepare copper standards within the linear range (2 - 500 $\mu\text{g l}^{-1}$), using working copper solution. Make all the dilutions using deionized distilled water.
- 8.2 Switch on the AAS and optimize instrumental parameters (refer Chapter 1.7.4).
- 8.3 Aspirate the sample blank (prepared by the procedure 9.1 - 9.19 replacing the sample with deionized distilled water).
- 8.4 Set zero absorbance.
- 8.5 Aspirate standards prepared by the procedure 9.1-9.19 replacing the sample with standards and record the absorbance.
- 8.6 Plot the calibration curve, absorbance versus copper concentration of standards.

9. Procedure

- 9.1 Measure a volume of (100 ml maximum) well mixed sample into a 125 ml beaker.
- 9.2 Adjust the volume to 100 ml with deionized distilled water, if necessary.
- 9.3 Add 5 ml of conc. HCl.
- 9.4 Heat the sample in a steam bath or on a hot plate until the volume has reduced to 15 to 20 ml (do not boil the sample).
- 9.5 Cool and filter the sample into 250 ml separatory funnel.
- 9.6 Wash the filter paper with distilled water into the funnel and adjust the volume approximately to 100 ml.
- 9.7 Add 2 drops of bromphenol blue indicator and mix well.
- 9.8 Adjust the pH until a blue color persists, by adding NaOH solution.
- 9.9 Add HCl (1+49) dropwise until the blue color disappears.
- 9.10 Add 2.5 ml of HCl (1+49) in excess.
- 9.11 Add 10 ml of APDC/chloroform reagent and shake for few minutes.
- 9.12 Plug the tip of the separatory funnel with cotton wool, allow the phases to separate and drain the chloroform layer into a 100 ml beaker.
- 9.13 Repeat the extraction with 10 ml of chloroform and drain the chloroform layer into the same beaker.
- 9.14 Evaporate the solution (chloroform layer) to near dryness in a water bath under a well ventilated fume hood.
- 9.15 Remove beaker from heat and allow residual solvent to evaporate without further heating.
- 9.16 Add 2 ml nitric acid dropwise and mix well.
- 9.17 Evaporate the solution to near dryness.

IRON mg l⁻¹

Atomic Absorption spectrophotometric

Lamp iron hollow cathode

Wavelength 248.3 nm

Fuel acetylene

Oxidant air

Flame oxidizing

Working Range 0.3 - 5 mg l⁻¹

Detection limit 0.02 mg l⁻¹

Precision at 435 µg l⁻¹ 42%
at 855 µg l⁻¹ 20%

Accuracy at 435 µg l⁻¹ 0.7%
at 855 µg l⁻¹ 1.8%

Interferences: Chemical interference resulted by low flame

1 Application

1.1 The method can be used for potable, fresh and saline waters and effluents

2. Summary of method

2.1 The sample is aspirated into the flame where the element is atomized and absorbs incoming radiation of the hollow cathode lamp

2.2 Attenuation of radiation is measured as absorbance

3. Sampling

3.1 Direct, filtered through 0.45 µm membrane filter paper.

4. Preservation

4.1 Preserve at 4 °C.

5. Apparatus

5.1 Atomic Absorption Spectrophotometer

6. Reagents, chemicals and gases

6.1 AAS standard iron solution or pure iron wire

6.2 Conc. nitric acid (ultra pure) [HNO₃]

6.3 Compressed air

6.4 Acetylene

7. Preparation of standards and reagents

- 7.1 Standard iron stock solution (1000 ppm). Obtain commercially available AAS standard or prepare manually as follows:
- 7.1.1 Weigh 1.000 g of pure iron wire.
 - 7.1.2 Dissolve in 5 ml ultra pure HNO₃, warming if necessary.
 - 7.1.3 When dissolution is complete, dilute up to 1 liter with deionized distilled water.
- 7.2 Working standard iron solution (10 ppm)
- 7.2.1 Prepare a 100 fold dilution of the stock solution using deionized distilled water.

8. Calibration

- 8.1 Prepare a series of standards using the working standard iron solution within the linear range (0.3 - 5 mg l⁻¹) as required.
- 8.2 Aspirate a blank consisting of the same nitric acid concentration as of the standards, prepared with distilled water and set zero absorbance.
- 8.3 Aspirate a standard iron solution and adjust aspiration rate of the nebulizer to obtain maximum response.
- 8.4 Aspirate the blank again and rezero the instrument.
- 8.5 Aspirate standards and record absorbances.
- 8.6 Plot the calibration curve, absorbance versus concentration of iron.

9. Procedure

- 9.1 Take a sample or an aliquot with appropriate dilution of 10 ml volume.
- 9.2 Aspirate the sample and record the absorbance.
- 9.3 Determine the concentration of iron from the curve.

10. Calculation

$$\text{Iron, mg l}^{-1} = C \times D$$

where; C = concentration of Fe from the curve
D = dilution factor

11. Alternative method

- Spectrophotometry with 2,2' bipyridine method (Fresenius, 1988)

NB: * The following wavelengths also may be used,
248.8 nm, 271.9 nm, 302.1 nm, 252.7 nm, 372.0 nm.

LEAD $\mu\text{g l}^{-1}$

Chelation/Extraction - AAS

Lamp: lead hollow cathode lamp

Wavelength: 283.3 nm

Fuel: acetylene

Oxidant: air

Working Range. 100 - 1000 $\mu\text{g l}^{-1}$

Precision : at 100 $\mu\text{g l}^{-1}$ 18 %
at 800 $\mu\text{g l}^{-1}$ 16 %

Accuracy. at 100 $\mu\text{g l}^{-1}$ -13.9 %
at 800 $\mu\text{g l}^{-1}$ -15.8 %

Interferences. excessive amounts of Bi, Th, and Sn

1. **Application**

1.1 The method is applicable to potable, fresh and saline waters and effluents

2. **Summary of method**

2.1 Lead is chelated with ammonium pyrrolidine dithiocarbamate (APDC) and extracted with chloroform.

2.2 The extract is treated with nitric acid to destroy organic matter and is dissolved in hydrochloric acid.

2.3 The lead in the resulting solution is then determined by aspirating to AAS.

3. **Sampling**

3.1 Direct, filtered through 0.45 μm membrane filter paper.

4. **Preservation**

4.1 Acidified with nitric acid to $\text{pH} < 1$

5. **Apparatus**

5.1 Atomic Absorption Spectrophotometer

6. **Reagents, chemicals and gases**

6.1 Bromphenol blue

6.2 AAS standard lead solution or lead nitrate (analytical grade) $[\text{Pb}(\text{NO}_3)_2]$

6.3 Chloroform $[\text{CHCl}_3]$

- 6.4 Ethanol or Isopropanol [C_2H_5OH or C_3H_7OH]
- 6.5 Ammonium pyrrolidine dithiocarbamate (APDC) [$C_5H_8NS_2.NH_4$]
- 6.6 Conc. hydrochloric acid [HCl]
- 6.7 Conc. nitric acid (ultra pure) [HNO_3]
- 6.8 Sodium hydroxide [NaOH]
- 6.9 Carbon disulfide [CS_2]
- 6.10 Compressed air
- 6.11 Acetylene

7 Preparation of standards and reagents

- 7.1 Bromphenol blue indicator solution (1 g l^{-1})
 - 7.1.1 Dissolve 0.1 g of bromphenol blue in 100 ml of 50% ethanol or isopropanol.
- 7.2 Standard stock lead solution ($1\text{ ml} \equiv 200\ \mu\text{g Pb}$) Obtain commercially available AAS standard or prepare manually as follows.
 - 7.2.1 Dissolve 0.3198 g of lead nitrate in a small amount of deionized distilled water containing 1 ml of nitric acid and dilute to 1 liter
- 7.3 Intermediate lead solution ($1\text{ ml} \equiv 2\ \mu\text{g Pb}$)
 - 7.3.1 Dilute 10 ml of stock solution (7.2) and 1 ml of nitric acid to 1 liter with deionized distilled water.
- 7.4 Working standard lead solution ($1\text{ ml} \equiv 0.2\ \mu\text{g Pb}$)
 - 7.4.1 Dilute 10 ml of intermediate lead solution (7.3) and 1 ml of nitric acid to 100 ml with deionized distilled water
- 7.5 Hydrochloric acid (1+2)
 - 7.5.1 Add 1 volume of HCl to 2 volumes of deionized distilled water.
- 7.6 Hydrochloric acid (1+49)
 - 7.6.1 Add 1 volume of HCl to 49 volumes of deionized distilled water
- 7.7 APDC/chloroform reagent
 - 7.7.1 Add 36 ml of APDC to 1 liter of chloroform.
 - 7.7.2 Cool the solution and add 30 ml of carbon disulfide in small portions.
 - 7.7.3 Dilute to 2 liters with chloroform
- 7.8 Sodium hydroxide solution (100 g l^{-1})
 - 7.8.1 Dissolve 100 g of sodium hydroxide in distilled water and dilute to 1 liter.

8 Calibration

- 8.1 Prepare lead standards within the linear range ($100 - 1000\ \mu\text{g l}^{-1}$), using working lead solution. Make all the dilutions using deionized distilled

- water.
- 8.2 Switch on the AAS and optimize instrumental parameters (refer Chapter 1.7.4).
 - 8.3 Aspirate the sample blank (prepared by the procedure 9.1 - 9.19 replacing the sample with distilled water).
 - 8.4 Set zero absorbance.
 - 8.5 Aspirate standards prepared by the procedure 9.1 to 9.19 replacing the sample with standards and record the absorbance.
 - 8.6 Plot the calibration curve, absorbance versus concentration of lead standards.

9 Procedure

- 9.1 Measure a volume of (100 ml maximum) well mixed sample into a 125 ml beaker.
- 9.2 Adjust the volume to 100 ml with distilled water, if necessary.
- 9.3 Add 5 ml of conc. HCl.
- 9.4 Heat the sample on a steam bath or a hot plate until the volume has reduced to 15 to 20 ml (do not boil the sample).
- 9.5 Cool and filter the sample into 250 ml separatory funnel.
- 9.6 Wash the filter paper with distilled water in to the funnel and adjust the volume approximately to 100 ml.
- 9.7 Add 2 drops of bromphenol blue indicator and mix well
- 9.8 Adjust the pH until a blue color persists by adding NaOH solution
- 9.9 Add HCl (1+49) dropwise until the blue color disappears.
- 9.10 Add 2.5 ml of HCl (1+49) in excess.
- 9.11 Add 10 ml of APDC/chloroform reagent and shake for few minutes.
- 9.12 Plug the tip of the separatory funnel with cotton wool, allow the phases to separate and drain the chloroform layer into a 100 ml beaker
- 9.13 Repeat the extraction with 10 ml of chloroform and drain the chloroform layer into the same beaker
- 9.14 Evaporate the solution (chloroform layer) to near dryness in a water bath under a well ventilated fume hood.
- 9.15 Remove beaker from heat and allow residual solvent to evaporate without further heating.
- 9.16 Add 2 ml nitric acid dropwise and mix well.
- 9.17 Evaporate the solution to near dryness.
- 9.18 Add 2 ml of HCl (1+2) to the beaker, and heat while swirling for 1 minute.
- 9.19 Cool and transfer the solution quantitatively to a 10 ml volumetric flask and adjust the volume with distilled water.

- 9.20 Aspirate the sample to the flame and record the absorbance.
9.21 Determine the concentration of lead from the calibration curve.

10. **Calculation**

$$\text{Lead, } \mu\text{gl}^{-1} = \frac{C \times 100}{V}$$

where; V = volume of original sample taken in 9.1 (ml)
C = lead concentration from the calibration curve

11. **Alternative method**

- Spectrophotometric with dithizone method (APHA, 1989)

NB: * APDC/chloroform reagent and lead salts are highly toxic and carbon disulfide is highly flammable.

MAGNESIUM mg^l⁻¹

Atomic Absorption spectrophotometric
Lamp: magnesium hollow cathode
Wavelength 285.2 nm
Fuel acetylene
Oxidant: air
Flame: oxidizing

Working Range: 0.02 - 0.5 mg^l⁻¹

Detection limit 0.0005 mg^l⁻¹

Precision at 0.2 mg^l⁻¹ 10.5%

Accuracy at 0.2 mg^l⁻¹ 6.3%

Interferences: Chemical interference resulted by low flame

1. Application

The method is applicable to potable, fresh and saline waters and effluents.

2. Summary of method

- 2.1 The sample is aspirated into the flame of AAS where the element is atomized and absorbs incoming radiation of the hollow cathode lamp.
- 2.2 Attenuation of radiation is measured as absorbance.

3. Sampling

- 3.1 Direct, filtered through 0.45 μm membrane filter paper.

4. Preservation

- 4.1 Preserve at 4 °C

5. Apparatus

- 5.1 Atomic Absorption Spectrophotometer

6. Reagents, chemicals and gases

- 6.1 AAS standard magnesium solution or magnesium oxide (analytical grade) [MgO]
- 6.2 Conc. nitric acid (ultra pure) [HNO₃]
- 6.3 Lanthanum oxide [La₂O₃]
- 6.4 Compressed air

6.5 Acetylene

7. Preparation of standards and reagents

7.1 Standard magnesium stock solution (500 mg l⁻¹ Mg) Obtain commercially available AAS standard or prepare manually as follows:

7.1.1 Dissolve 0.829 g of magnesium oxide in 10 ml of ultra pure HNO₃ and dilute to 1 liter with deionized distilled water.

7.2 Working standard magnesium solution (5 mg l⁻¹ Mg)

7.2.1 Prepare a 100 fold dilution of the stock solution (7.1) with deionized distilled water.

7.3 Lanthanum chloride solution

7.3.1 Dissolve 29 g of lanthanum oxide slowly in 250 ml conc HCl by adding small portion at a time.

7.3.2 Dilute to 500 ml with deionized distilled water.

8. Calibration

8.1 Prepare magnesium standards within the linear range (0.02 - 0.5 mg l⁻¹), using working magnesium solution. Make all the dilutions using deionized distilled water.

8.2 To each 10 ml standard add 1 ml of lanthanum chloride solution and mix well.

8.3 Switch on the AAS and optimize instrumental parameters (refer Chapter 1.7.4).

8.4 Aspirate the sample blank (i.e. distilled water with 1 ml of lanthanum chloride).

8.5 Set zero absorbance.

8.6 Aspirate standards and record the absorbance.

8.7 Plot the calibration curve, absorbance versus concentration of magnesium

9. Procedure

9.1 Take 10 ml of the sample or an aliquot of the sample diluted to 10 ml.

9.2 Add 1.0 ml of the lanthanum chloride solution and mix well.

9.3 Aspirate the sample and record the absorbance.

9.4 Determine the concentration of magnesium from calibration curve.

10. Calculation

$$\text{Magnesium, mg l}^{-1} = C \times D$$

where; C = concentration of Mg from the curve
D = dilution factor

11. **Alternative method**

- EDTA titration (APHA, 1989)

- NB:*
- * The interferences caused by aluminum at concentrations $> 2 \text{ mg l}^{-1}$ are masked by addition of lanthanum.
 - * The wavelength 202.5 nm also may be used.

MANGANESE mg l^{-1}

Atomic Absorption spectrophotometric

Lamp manganese hollow cathode

Wavelength: 279.5 nm

Fuel: acetylene

Oxidant: air

Flame oxidizing

Working Range: 0.1 - 3 mg l^{-1}

Detection limit 0.01 mg l^{-1}

Precision at 104 $\mu\text{g l}^{-1}$ 30%
at 432 $\mu\text{g l}^{-1}$ 16%

Accuracy at 104 $\mu\text{g l}^{-1}$ -2.1%
at 432 $\mu\text{g l}^{-1}$ +1.5%

Interferences: Chemical interference resulted by low flame

1. Application

1.1 The method is applicable for potable, fresh and saline waters and effluents.

2. Summary of method

2.1 The sample is aspirated into the flame of AAS where the element is atomized and absorbs incoming radiation of the hollow cathode lamp.

2.2 Attenuation of radiation is measured as absorbance.

3. Sampling

3.1 Direct, filtered through 0.45 μm membrane filter paper.

4. Preservation

4.1 Preserve at 4 $^{\circ}\text{C}$

5. Apparatus

5.1 Atomic Absorption Spectrophotometer

6. Reagents, chemicals and gases

6.1 AAS standard manganese solution or pure manganese metal

6.2 Conc. nitric acid (ultra pure) [HNO_3]

6.3 Conc. hydrochloric acid [HCl]

- 6.4 Compressed air
- 6.5 Acetylene

7. Preparation of standards and reagents

- 7.1 Standard manganese stock solution (1000 mg^l⁻¹ Mn). Obtain commercially available AAS standard or prepare manually as follows:
 - 7.1.1 Weigh 1.000 g of manganese metal.
 - 7.1.2 Dissolve in 10 ml of ultra pure HNO₃.
 - 7.1.3 When dissolution is complete, dilute to 1 liter with 1% HCl.
- 7.2 Working standard manganese solution (10 mg^l⁻¹ Mn)
 - 7.2.1 Prepare a 100 fold dilution of the stock solution with deionized distilled water.

8 Calibration

- 8.1 Prepare a series of manganese standards within the range required (0.1 - 3 mg^l⁻¹), using the working standard manganese solution (7.2).
- 8.2 Aspirate a blank consisting of the same nitric acid concentration as of the standards, prepared in distilled water and set zero absorbance
- 8.3 Aspirate a standard solution and adjust aspiration rate of the nebulizer to obtain maximum response.
- 8.4 Aspirate the blank again and rezero the instrument.
- 8.5 Aspirate standards and record absorbances.
- 8.6 Plot the calibration curve, absorbance versus concentration of manganese.

9. Procedure

- 9.1 Take a sample or an aliquot with appropriate dilution of 10 ml volume.
- 9.2 Aspirate the sample and record the absorbance.
- 9.3 Determine the concentration of manganese from the curve.

10. Calculation

$$\text{Manganese, mg l}^{-1} = C \times D$$

where; C = concentration of Mn from the curve
D = dilution factor

11. **Alternative method**

- Spectrophotometry with persulfate method (APHA, 1989)

NB: * The wavelength 403.1 nm also may be used.

NICKEL $\mu\text{g l}^{-1}$

Chelation/Extraction - AAS
Lamp: nickel hollow cathode
Wavelength: 232.0 nm
Fuel: acetylene
Oxidant: air

Working Range. 10 - 1000 $\mu\text{g l}^{-1}$

Precision: at 399.4 $\mu\text{g l}^{-1}$ 9%
at 794.4 $\mu\text{g l}^{-1}$ 17%

Accuracy: at 399.4 $\mu\text{g l}^{-1}$ -0.2%
at 794.4 $\mu\text{g l}^{-1}$ -11.7%

Interferences. Cd, Pb, Cu, Zn, Co, Cr > 10 mg l^{-1}

1. Application

1.1 Method is applicable to potable, fresh and saline waters and effluents.

2. Summary of method

2.1 Nickel is chelated with ammonium pyrrolidine dithiocarbamate (APDC) and extracted into chloroform.

2.2 The extract is treated with nitric acid to destroy organic matter and is dissolved in hydrochloric acid.

2.3 The nickel in the resulting solution is then determined by aspirating to AAS.

3. Sampling

3.1 Direct, filtered through 0.45 μm membrane filter paper.

4. Preservation

4.1 Acidified with nitric acid to $\text{pH} < 1$

5. Apparatus

5.1 Atomic Absorption Spectrophotometer

6. Reagents, chemicals and gases

6.1 Bromphenol blue

6.2 AAS standard nickel solution or nickelous nitrate (analytical grade)

- [Ni(NO₃)₂·6H₂O]
- 6.3 Chloroform [CHCl₃]
- 6.4 Ammonium pyrrolidine dithiocarbamate (APDC) [C₅H₈NS₂·NH₄]
- 6.5 Ethanol or Isopropanol [C₂H₅OH or C₃H₇OH]
- 6.6 Conc. hydrochloric acid [HCl]
- 6.7 Conc. nitric acid (ultra pure) [HNO₃]
- 6.8 Sodium hydroxide [NaOH]
- 6.9 Compressed air
- 6.10 Acetylene

7. Preparation of standards and reagents

- 7.1 Bromphenol blue indicator solution (1 gl⁻¹)
 - 7.1.1 Dissolve 0.1 g of bromphenol blue in 100 ml of 50% ethanol or isopropanol.
- 7.2 Standard nickel stock solution (1 ml ≡ 200 μg Ni). Obtain commercially available AAS standard or prepare manually as follows:
 - 7.2.1 Dissolve 0.9906 g of nickelous nitrate in deionized distilled water containing 1 ml of nitric acid.
 - 7.2.2 Dilute to 1 liter with deionized distilled water.
- 7.3 Working standard nickel solution (1 ml ≡ 2 μg Ni)
 - 7.3.1 Dilute 10 ml of nickel stock solution (7.2) and 1 ml of nitric acid to 1 liter with deionized distilled water.
- 7.4 Hydrochloric acid (1+2)
 - 7.4.1 Add 1 volume of HCl to 2 volumes of deionized distilled water.
- 7.5 Hydrochloric acid (1+49)
 - 7.5.1 Add 1 volume of HCl to 49 volumes of deionized distilled water.
- 7.6 APDC/chloroform reagent
 - 7.6.1 Add 36 ml of APDC to 1 liter of chloroform.
 - 7.6.2 Cool the solution and add 30 ml of carbon disulfide in small portions.
 - 7.6.3 Dilute to 2 liters with chloroform.
- 7.7 Sodium hydroxide solution (100 gl⁻¹)
 - 7.7.1 Dissolve 100 g of sodium hydroxide in distilled water and dilute to 1 liter.

8. Calibration

- 8.1 Prepare nickel standards using working nickel solution within the linear range (10 - 1000 μgl⁻¹). Make all the dilutions using deionized distilled water.

- 8.2 Switch on the AAS and optimize instrumental parameters (refer Chapter 1.7.4).
- 8.3 Aspirate the sample blank (prepared by the procedure 9.1 - 9.19 replacing the sample with deionized distilled water).
- 8.4 Set zero absorbance.
- 8.5 Aspirate standards prepared by the procedure 9.1 - 9.19 replacing the sample with standards and record the absorbance.
- 8.6 Plot the calibration curve, absorbance versus concentration of nickel standards.

9. Procedure

- 9.1 Measure a volume of (100 ml maximum) well mixed sample into a 125 ml beaker.
- 9.2 Adjust the volume to 100 ml with distilled water, if necessary.
- 9.3 Add 5 ml of conc. HCl.
- 9.4 Heat the sample in a steam bath or on a hot plate until the volume has reduced to 15 to 20 ml (do not boil the sample).
- 9.5 Cool and filter the sample into 250 ml separatory funnel.
- 9.6 Wash the filter paper with distilled water into the funnel and adjust the volume approximately to 100 ml.
- 9.7 Add 2 drops of bromphenol blue indicator and mix well.
- 9.8 Adjust the pH until a blue color persists, by adding NaOH solution.
- 9.9 Add HCl (1+49) dropwise until the blue color disappears.
- 9.10 Add 2.5 ml of HCl (1+49) in excess.
- 9.11 Add 10 ml of APDC/chloroform reagent and shake for few minutes.
- 9.12 Plug the tip of the separatory funnel with cotton wool, allow the phases to separate and drain the chloroform layer into a 100 ml beaker.
- 9.13 Repeat the extraction with 10 ml of chloroform and drain the chloroform layer into the same beaker.
- 9.14 Evaporate the solution (chloroform layer) to near dryness in a water bath under a well ventilated fume hood.
- 9.15 Remove beaker from heat and allow residual solvent to evaporate without further heating.
- 9.16 Add 2 ml nitric acid dropwise and mix well.
- 9.17 Evaporate the solution to near dryness.
- 9.18 Add 2 ml of HCl (1+2) to the beaker, and heat while swirling for 1 minute.
- 9.19 Cool and transfer the solution quantitatively to a 10 ml volumetric flask and adjust the volume with deionized distilled water.
- 9.20 Aspirate the sample to the flame and record the absorbance.

9.21 Determine the concentration of nickel from the calibration curve.

10. **Calculation**

$$\text{Nickel, } \mu\text{gl}^{-1} = \frac{C \times 100}{V}$$

where; V = volume of original sample taken in 9.1 (ml)
C = concentration of nickel from the calibration curve

11. **Alternative method**

- Spectrophotometry with dimethyl glyoxime method (APHA, 1989)

NB: * APDC/chloroform reagent is highly toxic and carbon disulfide is highly flammable.

POTASSIUM mg l⁻¹

Atomic Emission Spectrophotometric

Wavelength: 766.5 nm

Fuel: acetylene

Oxidant: air

Flame: slightly oxidizing

Working Range >0.1 mg l⁻¹

Detection limit: 0.01 mg l⁻¹

Precision at 3.1 mg l⁻¹ 15.5%

Accuracy: at 3.1 mg l⁻¹ 2.3%

Interferences: Ca - If Ca:K > 10:1, Mg - If Mg:K > 100:1; Na - If Na:K > 5:1

1. Application

1.1 The method is applicable for potable, fresh and saline waters and effluents.

2. Summary of method

2.1 The sample is aspirated into the flame of AAS where the element is atomized and excited.

2.2 The radiation emitted during de-excitation process is measured.

3. Sampling

3.1 Direct, filtered through 0.45 μm membrane filter paper.

4. Preservation

4.1 Preserve at 4 °C.

5. Apparatus

5.1 Atomic Absorption Spectrophotometer with emission mode.

6. Reagents, chemicals and gases

6.1 AAS standard potassium solution or potassium chloride (analytical grade) [KCl]

6.2 Compressed air

6.3 Acetylene

7. **Preparation of standards and reagents**

- 7.1 Standard potassium stock solution (100 mg^l⁻¹ K). Obtain commercially available AAS standard or prepare manually as follows:
7.1.1 Dissolve 0.1907 g of potassium chloride (dried at 110 °C) in deionized distilled water.
7.1.2 Make up to 1 liter.
7.2 Working standard potassium solution (10 mg^l⁻¹ K)
7.2.1 Prepare a 10 fold dilution of the stock solution (7.1) with deionized distilled water.

8. **Calibration**

- 8.1 Prepare a blank and potassium standards covering the working range by diluting the working standard potassium solution.
8.2 Aspirate the blank and set zero emission at 766.5 nm.
8.3 Starting with the highest calibration standard, measure the emission at the same wavelength.
8.4 Plot a calibration curve of atomic emission versus concentration of potassium.

9. **Procedure**

- 9.1 Measure emission of the samples at 766.5 nm.
9.2 Determine concentration from the calibration curve.

10. **Calculation**

$$\text{Potassium, mg l}^{-1} = C \times D$$

where; C = concentration of K from the curve
D = dilution factor

11. **Alternative method**

- Flame photometric method (APHA, 1989)

SODIUM mg l⁻¹

Atomic Emission Spectrophotometric

Wavelength. 589.6 nm

Fuel acetylene

Oxidant: air

Flame: oxidizing

Working Range: >0.1 mg l⁻¹

Detection limit: 0.01 mg l⁻¹

Precision: at 19.9 mg l⁻¹ 17.3%

Accuracy: at 19.9 mg l⁻¹ 4.0%

Interferences: K - If K Na >5:1; Ca- If Ca Na >10:1

1. Application

1.1 The method is applicable to potable, fresh and saline waters and effluents.

2. Summary of method

2.1 The sample is aspirated into the flame of AAS where the element is atomized and excited.

2.2 The radiation emitted during de-excitation process is measured.

3. Sampling

3.1 Direct, filtered through 0.45 μm membrane filter paper.

4. Preservation

4.1 Preserve at 4 °C.

5. Apparatus

5.1 Atomic Absorption Spectrophotometer with emission mode

6. Reagents, chemicals and gases

6.1 AAS standard sodium solution or sodium chloride [NaCl]

6.2 Compressed air

6.3 Acetylene

7. Preparation of standards and reagents

- 7.1 Standard sodium stock solution (1000 mg l⁻¹ Na). Obtain commercially available AAS standard or prepare manually as follows:
 - 7.1.1 Dissolve 2.542 g sodium chloride (dried at 140 °C) in deionized distilled water and make up to 1000 ml.
- 7.2 Working standard sodium solution (10 mg l⁻¹ Na)
 - 7.2.1 Prepare a 100 fold dilution of the stock solution (7.1) with deionized distilled water.

8. Calibration

- 8.1 Prepare a blank and a series of sodium standards covering the working range by diluting the working standard sodium solution.
- 8.2 Aspirate the blank and set zero emission at 589.6 nm.
- 8.3 Starting with the highest calibration standard, measure emission at the same wavelength.
- 8.4 Plot a calibration curve of atomic emission versus concentration of sodium.

9. Procedure

- 9.1 Measure emission of the samples at 589.6 nm.
- 9.2 Determine concentration from the calibration curve.

10. Calculation

$$\text{Sodium, mg l}^{-1} = C \times D$$

where; C = concentration of Na from the curve
D = dilution factor

11. Alternative method

- Flame photometric method (APHA, 1989)

ZINC $\mu\text{g l}^{-1}$

Chelation/Extraction - AAS

Lamp: zinc hollow cathode

Wavelength: 213.8 nm

Fuel: acetylene

Oxidant: air

Working Range: 1 - 200 $\mu\text{g l}^{-1}$

Precision : at 10 $\mu\text{g l}^{-1}$ 160%
at 170 $\mu\text{g l}^{-1}$ 14%

Accuracy: at 75.6 $\mu\text{g l}^{-1}$ +8.1%
at 172 $\mu\text{g l}^{-1}$ +7.5%

Interferences: Small quantities of Bi, Cd, Co, Cu, Au, Pb, Hg, Ni, Ag and Sn

1. Application

1.1 The method is applicable to potable, fresh and saline waters and effluents.

2. Summary of method

2.1 Zinc is chelated with ammonium pyrrolidine dithiocarbamate (APDC) and extracted into chloroform.

2.2 The extract is treated with nitric acid to destroy organic matter and is dissolved in hydrochloric acid.

2.3 The zinc in the resulting solution is then determined by aspirating to AAS.

3. Sampling

3.1 Direct, filtered through 0.45 μm membrane filter paper.

4. Preservation

4.1 Acidified with nitric acid to $\text{pH} < 1$.

5. Apparatus

5.1 Atomic Absorption Spectrophotometer

6. Reagents, chemicals and gases

6.1 Bromphenol blue

6.2 AAS standard zinc solution or zinc oxide (analytical grade) $[\text{ZnO}]$

6.3 Ammonium pyrrolidine dithiocarbamate (APDC) $[\text{C}_5\text{H}_8\text{NS}_2\cdot\text{NH}_4]$

- 6.4 Chloroform [CHCl_3]
- 6.5 Carbon disulfide [CS_2]
- 6.6 Ethanol or Isopropanol [$\text{C}_2\text{H}_5\text{OH}$ or $\text{C}_3\text{H}_7\text{OH}$]
- 6.7 Conc. hydrochloric acid [HCl]
- 6.8 Conc. nitric acid (ultra pure) [HNO_3]
- 6.9 Sodium hydroxide [NaOH]
- 6.10 Compressed air
- 6.11 Acetylene

7. Preparation of standards and reagents

- 7.1 Bromphenol blue indicator solution (1 g^{-1})
 - 7.1.1 Dissolve 0.1 g of bromphenol blue in 100 ml of 50% ethanol or isopropanol.
- 7.2 Standard zinc stock solution ($1 \text{ ml} \equiv 1 \text{ mg Zn}$). Obtain commercially available AAS standard or prepare manually as follows:
 - 7.2.1 Dissolve 1.245 g of zinc oxide in a mixture of 10 ml of nitric acid and 10 ml of deionized distilled water and dilute to 1 liter.
- 7.3 Intermediate zinc solution ($1 \text{ ml} \equiv 0.1 \text{ mg Zn}$)
 - 7.3.1 Dilute 100 ml of stock solution (7.2) and 1 ml of nitric acid to 1 liter with deionized distilled water.
- 7.4 Working standard zinc solution ($1 \text{ ml} \equiv 1 \mu\text{g Zn}$)
 - 7.4.1 Dilute 10 ml of zinc intermediate solution (7.3) and 1 ml of nitric acid to 1 liter with deionized distilled water.
- 7.5 Hydrochloric acid (1+2)
 - 7.5.1 Add 1 volume of HCl to 2 volumes of deionized distilled water.
- 7.6 Hydrochloric acid (1+49)
 - 7.6.1 Add 1 volume of HCl to 49 volumes of deionized distilled water.
- 7.7 APDC/chloroform reagent
 - 7.7.1 Add 36 ml of APDC to 1 liter of chloroform.
 - 7.7.2 Cool the solution and add 30 ml of carbon disulfide in small portions.
 - 7.7.3 Dilute to 2 liters with chloroform.
- 7.8 Sodium hydroxide solution (100 g^{-1})
 - 7.8.1 Dissolve 100 g of sodium hydroxide in distilled water and dilute to 1 liter.

8. Calibration

- 8.1 Prepare zinc standards within the linear range ($10 - 1000 \mu\text{g}^{-1}$), using working zinc solution. Make all the dilutions using deionized distilled

- water.
- 8.2 Switch on the AAS and optimize instrumental parameters (refer Chapter 1.7.4).
 - 8.3 Aspirate the sample blank (prepared by the procedure 9.1 - 9.19 replacing the sample with deionized distilled water).
 - 8.4 Set zero absorbance.
 - 8.5 Aspirate standards prepared by the procedure 9.1 - 9.19 replacing the sample with standards and record the absorbance.
 - 8.6 Plot the calibration curve, absorbance versus concentration of zinc standards.

9. Procedure

- 9.1 Measure a volume of (100 ml maximum) well mixed sample into a 125 ml beaker.
- 9.2 Adjust the volume to 100 ml with deionized distilled water if necessary.
- 9.3 Add 5 ml of conc. HCl.
- 9.4 Heat the sample in a steam bath or on a hot plate until the volume has reduced to 15 to 20 ml (do not boil the sample).
- 9.5 Cool and filter the sample into 250 ml separatory funnel.
- 9.6 Wash the filter paper with distilled water in to the funnel and adjust the volume approximately to 100 ml.
- 9.7 Add 2 drops of bromphenol blue indicator and mix well.
- 9.8 Adjust the pH until a blue color persists, by adding NaOH solution.
- 9.9 Add HCl (1+49) dropwise until the blue color disappears.
- 9.10 Add 2.5 ml of HCl (1+49) in excess.
- 9.11 Add 10 ml of APDC/chloroform reagent and shake for few minutes.
- 9.12 Plug the tip of the separatory funnel with cotton wool, allow the phases to separate and drain the chloroform layer into a 100 ml beaker.
- 9.13 Repeat the extraction with 10 ml of chloroform and drain the chloroform layer into the same beaker.
- 9.14 Evaporate the solution (chloroform layer) to near dryness in a water bath under a well ventilated fume hood.
- 9.15 Remove beaker from heat and allow residual solvent to evaporate without further heating.
- 9.16 Add 2 ml nitric acid dropwise and mix well.
- 9.17 Evaporate the solution to near dryness.
- 9.18 Add 2 ml of HCl (1+2) to the beaker, and heat while swirling for 1 minute.
- 9.19 Cool and transfer the solution quantitatively to a 10 ml volumetric flask and adjust the volume with deionized distilled water.

- 9.20 Aspirate the sample to the flame and record the absorbance.
9.21 Determine the concentration of zinc from the calibration curve.

10. **Calculation**

$$\text{Zinc, } \mu\text{gl}^{-1} = \frac{C \times 100}{V}$$

where; V = volume of original sample in ml
C = concentration of zinc from the calibration curve

11. **Alternative method**

- Spectrophotometry with dithiozone method (APHA, 1989)

NB: * APDC/chloroform reagent is highly toxic and carbon disulfide is highly flammable.

5.4 Non-metallic constituents

ALKALINITY mg l^{-1} as CaCO_3

Titrimetric

Working Range: All concentration ranges

Precision: at 122 mg l^{-1} as CaCO_3 - 2.5%

Interferences: Salts of weak organic and inorganic acids, oil and grease

1. Application

1.1 The method is applicable to potable, fresh and saline waters and effluents.

2. Summary of method

2.1 An unfiltered and unaltered sample is titrated with a dilute acid to an electrometrically determined end point of pH 4.5.

3. Sampling

3.1 Direct, unfiltered

4. Preservation

4.1 No preservation, immediate measurement

5. Apparatus

5.1 pH meter (can be read to 0.05 pH units)

5.2 Magnetic stirrer

5.3 Pipettes, flasks and other standard glassware

5.4 Burettes (50, 25 and 10 ml)

6. Reagents and chemicals

6.1 Sodium carbonate [Na_2CO_3] (analytical grade)

6.2 Conc. sulfuric or con. hydrochloric [H_2SO_4 or HCl] (analytical grade)

7. Preparation of standards and reagents

- 7.1 Sodium carbonate solution (0.05 N)
 - 7.1.1 Dissolve 2.5 ± 0.20 g sodium carbonate (dried at 250°C for 4 hours and cooled in a desiccator) in a 1 liter of deionized distilled water.
- 7.2 Standard acid (0.1 N); (should be used for alkalinity > 20 mg l^{-1} as CaCO_3)
 - 7.2.1 Dilute 3.0 ml conc. H_2SO_4 or 8.3 ml conc. HCl to 1 liter with distilled water.
 - 7.2.2 Standardize with 0.05 N standard sodium carbonate solution (as given in the procedure 8.1).
- 7.3 Standard acid (0.02 N); (should be used for alkalinity < 20 mg l^{-1} as CaCO_3)
 - 7.3.1 Dilute 200 ml of 0.1 N standard acid to 1 liter with distilled water.
 - 7.3.2 Standardize with 0.05 N sodium carbonate solution (as given in the procedure 8.1).

8. Procedure

- 8.1 Standardization of acid
 - 8.1.1 Take 40 ml of 0.05 N sodium carbonate solution and add about 60 ml distilled water.
 - 8.1.2 Titrate with the acid potentiometrically to pH of about 5.
 - 8.1.3 Rinse the electrode into the beaker.
 - 8.1.4 Cover with a watch glass and boil the solution gently for 3 to 5 minutes.
 - 8.1.5 Cool to room temperature.
 - 8.1.6 Rinse cover glass into the beaker.
 - 8.1.7 Continue the titration to the pH inflection point.
 - 8.1.8 Calculate normality.
- 8.2 Potentiometric titration for high alkalinity (> 20 mg l^{-1})
 - 8.2.1 Fill a sufficiently large volume of titrant (> 20 ml) in a 50 ml burette
 - 8.2.2 Place sample in flask by pipetting with pipette tip near bottom of the flask (use a measured volume of the sample sufficient enough to cover the sensing elements of the electrode).
 - 8.2.3 Measure pH of the sample.
 - 8.2.4 Titrate with standard acid (0.1 N) while stirring to pH 4.5.
 - 8.2.5 Record volume of the titrant.
- 8.3 Potentiometric titration of low alkalinity (< 20 mg l^{-1})
 - 8.3.1 For alkalinity of < 20 mg l^{-1} , titrate 100 to 200 ml of sample as in

- 8.2 using a 10 ml micro burette and 0.02 N acid solution.
- 8.3.2 Stop titration at pH in the range of 4.3 - 4.7.
- 8.3.3 Record volume and exact pH.
- 8.3.4 Very carefully, add titrant to lower the pH exactly by 0.3 pH units and record volume again.

9. Calculation

9.1

$$\text{Normality of acid, } \text{eql}^{-1} = \frac{A \times V_1}{53.00 \times V_2}$$

where; A = Na₂CO₃ weighed into 1 liter
 V₁ = volume of Na₂CO₃ solution in ml
 V₂ = volume of acid used to inflection point in ml

9.2 For high alkalinity,

$$\text{Alkalinity, } \text{mgl}^{-1} \text{ as CaCO}_3 = \frac{V \times N \times 50,000}{\text{ml of sample}}$$

where; V = volume of standard acid in ml
 N = normality of acid

9.3 For low alkalinity,

$$\text{Alkalinity, } \text{mgl}^{-1} \text{ as CaCO}_3 = \frac{(2V_1 - V_2) \times N \times 50,000}{\text{ml of sample}}$$

where; V₁ = ml titrant to first recorded pH
 V₂ = total ml titrant to reach pH 0.3 units lower
 N = normality of acid

10. Alternative method

- Nomographic method (APHA, 1989)

Working Range. 1.5 - 250 mg l^{-1} Cl^{-}

Precision . at 8 mg l^{-1} 10%
at 250 mg l^{-1} 1.6%

Accuracy: at 8 mg l^{-1} -6.13%
at 250 mg l^{-1} -0.80%

Interferences. Orthophosphate > 200 mg l^{-1} , Polyphosphate > 25 mg l^{-1}

1. Application

1.1 The method is applicable to potable and fresh waters and effluents.

2. Summary of method

2.1 The chloride ion reacts with the silver ion before any silver chromate formation, due to the lower solubility of silver chloride.

2.2 The potassium chromate indicator reacts with excess silver ion at the end point forming a red silver chromate precipitate.

3. Sampling

3.1 Direct, filtered through 0.45 μm membrane filter paper.

4. Preservation

4.1 Store at 4 $^{\circ}\text{C}$

5. Apparatus

5.1 Standard laboratory glassware for titration

5.2 Burette, 25 ml capacity

6. Reagents and chemicals

6.1 Silver nitrate [AgNO_3] (analytical grade)

6.2 Hydrogen peroxide (30%) [H_2O_2]

6.3 Potassium chromate [K_2CrO_4]

6.4 Sodium chloride [NaCl] (analytical grade)

6.5 Sodium hydroxide [NaOH]

6.6 Conc. sulfuric acid [H₂SO₄]

7. Preparation of standards and reagents

7.1 Potassium chromate indicator solution

- 7.1.1 Dissolve 50 g of potassium chromate in 100 ml distilled water.
- 7.1.2 Add silver nitrate (7.3) until a slight red precipitate is produced.
- 7.1.3 Allow the solution to stand at least 24 hours in a dark place.
- 7.1.4 Filter to remove the precipitate.
- 7.1.5 Dilute to 1 liter with distilled water.

7.2 Standard sodium chloride solution (0.025 N)

- 7.2.1 Dry several grams of sodium chloride for 1 hour at 600 °C .
- 7.2.2 Dissolve 1.4613 g of the dried salt in deionized distilled water and dilute to 1 liter.

7.3 Standard silver nitrate solution (0.025 N)

- 7.3.1 Dissolve 4.2473 g of silver nitrate (crushed and dried to constant weight at 40 °C) in deionized distilled water and dilute to 1 liter.
- 7.3.2 Standardize against the sodium chloride solution (follow the procedure 8.1 to 8.6).

7.4 Sodium hydroxide solution (10 gl⁻¹)

- 7.4.1 Dissolve 10 g of sodium hydroxide in distilled water and dilute to 1 liter.

7.5 H₂SO₄ (1+19)

- 7.5.1 Add one volume of conc. H₂SO₄ acid to 19 volumes of distilled water, while mixing. (PRECAUTION: keep the diluting vessel in a water bath and add acid very slowly.)

8. Procedure

- 8.1 Pour volumetrically 50.0 ml or less of the sample containing not more than 20 nor less than 0.25 mg of chloride ion into a conical flask.
- 8.2 If sulfite ion is present, add 0.5 ml of hydrogen peroxide to the sample, mix well and let stand for 1 minute.
- 8.3 Dilute approximately to 50 ml with deionized distilled water if necessary
- 8.4 Adjust the pH to 8.3 using H₂SO₄ (1+19) or NaOH (10 gl⁻¹).
- 8.5 Add approximately 1.0 ml of K₂CrO₄ indicator and mix well.
- 8.6 Add standard silver nitrate solution dropwise from a 25 ml burette until the appearance of a permanent brick-red colour.
- 8.7 Repeat the procedure from 8.1 to 8.6 using exactly one half as much of the original sample, diluted to 50 ml with deionized distilled water.

9. **Calculation**

$$Cl^{-}, mg l^{-1} = \frac{[(V_1 - V_2) \times N \times 70,906]}{V_3}$$

where; V_1 = standard silver nitrate solution added in titration 8.6 (ml)
 V_2 = standard silver nitrate solution added in titration 8.7 (ml)
 N = normality of standard silver nitrate solution
 V_3 = original sample in the 50 ml test specimen prepared in 8.1 (ml)

10. **Alternative method**

- Spectrophotometry with ferricyanide method (USEPA, 1983)

NB: * Compounds which precipitate at pH 8.3 may cause error.
* Sulfite and objectionable colour or turbidity must be eliminated before the titration.

CYANIDE mg l^{-1}

Spectrophotometric
Wavelength: 578 nm
Path length: 1 cm

Working Range: 0.02 - 1 mg l^{-1}

Precision at 0.06 mg l^{-1} 8%
at 0.62 mg l^{-1} 15%

Accuracy: at 0.28 mg l^{-1} 85%
at 0.62 mg l^{-1} 102%

Interferences: Fatty acids

1. Application

1.1 The method is applicable to potable, fresh and saline waters and effluents.

2. Summary of method

2.1 The cyanide is released from cyanide complexes as hydrocyanic acid and absorbed into sodium hydroxide solution.

2.2 The cyanide absorbed in the solution is converted to cyanogen chloride (CNCl) by reaction with chloramine-T.

2.3 CNCl is converted to a red blue dye by addition of pyridine-barbituric acid reagent and the colour intensity is measured spectrophotometrically.

3. Sampling

3.1 Direct, filtered through 0.45 μm membrane filter paper.

4. Preservation

4.1 Preserve by adding NaOH pellets or strong NaOH solution to raise sample pH to 12-12.5 and store in an airtight dark bottle in a cool place.

5. Apparatus

5.1 Reflux distillation apparatus (Fig. 5.1)

5.2 UV/Visible spectrophotometer

6. Reagents and chemicals

6.1 Sodium hydroxide [NaOH]

- 6.2 Sulfuric acid [H_2SO_4] (1+1)
- 6.3 Magnesium chloride [$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$]
- 6.4 Lead carbonate [PbCO_3]

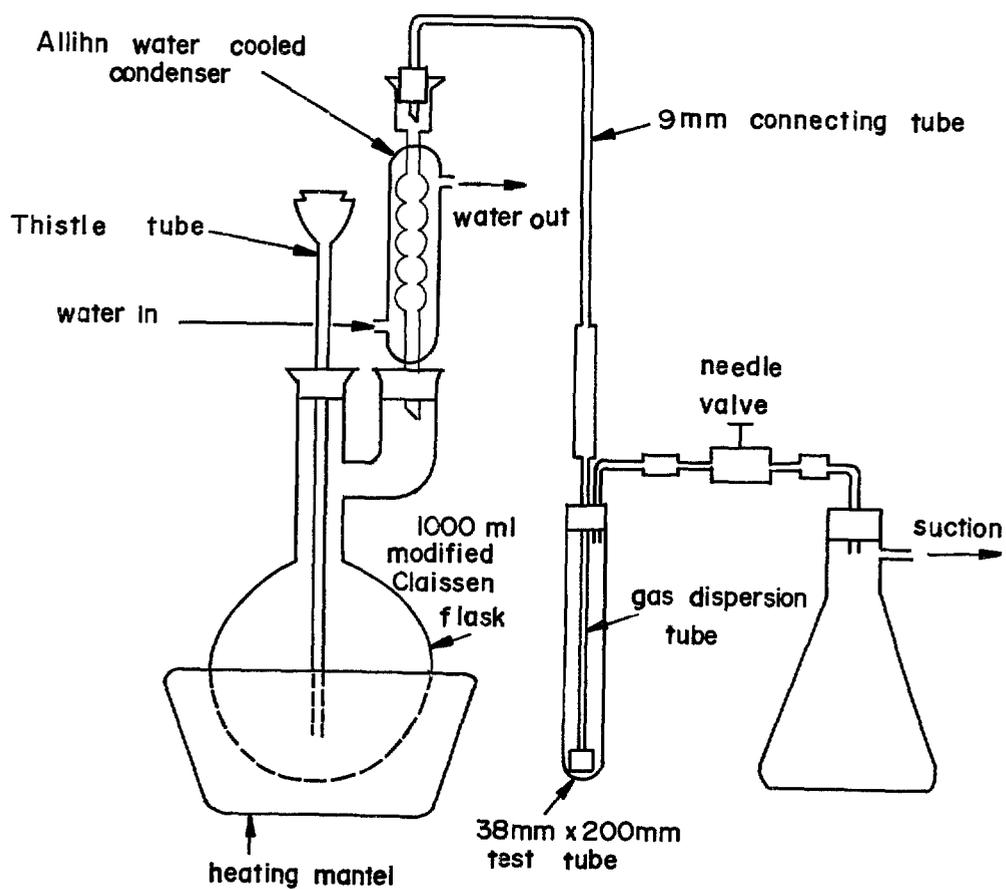


Fig. 5.1 Reflux distillation apparatus for cyanide

- 6.5 Sulfamic acid [$\text{NH}_2\text{SO}_3\text{H}$]
- 6.6 Chloramine-T [$\text{C}_6\text{H}_4\text{SO}_2\text{NCINa}$]

- 6.7 Potassium cyanide [KCN] (analytical grade)
- 6.8 Silver nitrate [AgNO₃] (analytical grade)
- 6.9 Barbituric acid [C₄H₄N₂O₃]
- 6.10 Pyridine [C₅H₅N]
- 6.11 Conc. hydrochloric acid [HCl]
- 6.12 Sodium dihydrogen phosphate [NaH₂PO₄·H₂O]
- 6.13 p-Dimethyl-amino-benzalrhodanine [C₁₂H₁₂N₂OS₂]
- 6.14 Acetone [C₃H₆O]

7. Preparation of standards and reagents

- 7.1 NaOH solution (40 g l⁻¹)
 - 7.1.1 Dissolve 40 g NaOH in distilled water and dilute to 1 liter.
- 7.2 NaOH dilution solution
 - 7.2.1 Dissolve 1.6 g NaOH in 1 liter distilled water.
- 7.3 Magnesium chloride solution
 - 7.3.1 Dissolve 510 g of magnesium chloride in distilled water and dilute to 1 liter.
- 7.4 Chloramine-T solution (prepare weekly)
 - 7.4.1 Dissolve 1 g of chloramine-T powder in 100 ml distilled water and store in a refrigerator.
- 7.5 Pyridine/Barbituric acid reagent
 - 7.5.1 Place 15 g of barbituric acid in a 250 ml volumetric flask and add barely enough amount of distilled water to wet the acid.
 - 7.5.2 Add 75 ml of pyridine and mix well.
 - 7.5.3 Add 15 ml conc.HCl, mix and cool to room temperature.
 - 7.5.4 Dilute to the mark with distilled water and mix well (discard if a precipitate develops and re-prepare with fresh chemicals).
- 7.6 Phosphate buffer solution
 - 7.6.1 Dissolve 138 g of sodium dihydrogen phosphate in 1 liter distilled water (refrigerate).
- 7.7 Standard Silver nitrate solution (0.0192 N)
 - 7.7.1 Dissolve 3.27 g of silver nitrate in 1 liter deionized distilled water (1 ml ≡ 1 mg NO₃⁻).
- 7.8 Rhodanine indicator
 - 7.8.1 Dissolve 20 mg of p-dimethyl-amino-benzalrhodanine in 100 ml acetone.
- 7.9 Standard cyanide stock solution (1 ml ≡ 1 mg CN⁻)
 - 7.9.1 Dissolve approximately 1.6 g NaOH and 2.51 g potassium cyanide in 1 liter deionized distilled water.
 - 7.9.2 Standardize against standard silver nitrate solution as described in 9.1.

- 7.10 Standard intermediate cyanide solution (1 ml \equiv 100 $\mu\text{g CN}^-$)
 - 7.10.1 Dilute 100 ml of the stock solution to 1000 ml with deionized distilled water.
- 7.11 Working standard cyanide solution (1 ml \equiv 10 $\mu\text{g CN}^-$)
 - 7.11.1 Dilute 100 ml of the intermediate solution to 1000 ml with deionized distilled water.
 - 7.11.2 Store in a glass stoppered bottle.

8. Calibration

- 8.1 Prepare a series of 10 ml standards covering the range of 0.2-10 $\mu\text{g CN}$ using the working standard cyanide solution (use NaOH dilution solution for dilutions).
- 8.2 Treat the standards as given in the procedure 9.3.1 - 9.3.6.
- 8.3 Plot the absorbance of the standards against cyanide content in μg .

9. Procedure

- 9.1 Standardization of stock cyanide solution
 - 9.1.1 Take 25 ml of the stock cyanide solution into a conical flask.
 - 9.1.2 Add few drops of rhodanine indicator.
 - 9.1.3 Titrate with standard silver nitrate solution to the first change in colour from yellow to brownish pink.
 - 9.1.4 Calculate the CN^- concentration of the cyanide stock solution (in mg ml^{-1}).
- 9.2 Distillation of samples
 - 9.2.1 Set up the distillation system as shown in Fig. 5.1.
 - 9.2.2 Place 500 ml sample in the boiling flask and 10 ml NaOH solution in the absorbing tube (if necessary, dilute with deionized distilled water to obtain an adequate liquid depth in the absorbing tube).
 - 9.2.3 For samples containing sulfide ion, add 50 mg or more powdered lead carbonate to the absorbing tube to precipitate sulfide.
 - 9.2.4 Connect the boiling flask, condenser, absorber and the trap.
 - 9.2.5 Start a slow stream of air entering the boiling flask by adjusting the vacuum source (approximately 2 bubbles of air per second should enter the flask).
 - 9.2.6 Add 2 g of sulfamic acid through the air inlet tube and wash down with distilled water.
 - 9.2.7 Add 50 ml H_2SO_4 (1+1) through the inlet tube. Rinse tube with distilled water and let air mix the contents for 3 minutes.
 - 9.2.8 Add 20 ml magnesium chloride solution through the air inlet and wash down with distilled water.

- 9.2.9 Heat the solution to boiling, reflux for 1 hour, turn-off heat and continue the air flow for at least 15 minutes. After cooling disconnect the absorber and close off the vacuum source.
- 9.3.0 Rinse connecting tube between condenser and absorbing tube with deionized distilled water, add rinse water to the drained liquid in the absorbing tube and dilute to 250 ml in a volumetric flask.
- 9.3 Determination of cyanide
- 9.3.1 Take a portion of the absorption liquid obtained from distillation (9.3.0) or cyanide standard (10 ml) prepared in 8.1 and dilute to 20 ml with NaOH dilution solution.
- 9.3.2 Place this solution in a 50 ml volumetric flask and add 4 ml phosphate buffer solution.
- 9.3.3 Add 2 ml chloramine-T solution and mix well.
- 9.3.4 Immediately add 5 ml pyridine-barbituric acid solution and mix well.
- 9.3.5 Dilute to the mark with distilled water.
- 9.3.6 Measure the absorbance at 578 nm after 8 minutes (measurements should be taken before 15 minutes).

10. Calculation

$$CN^-, \text{ mg l}^{-1} = \frac{A \times V_1}{V_2 \times V_3}$$

- where;
- | | | |
|-------|---|---|
| A | = | $\mu\text{g CN}^-$ corresponding to absorbance of the sample (from the calibration curve) |
| V_1 | = | total volume of absorption solution from the distillation (ml) |
| V_2 | = | volume of original sample used in the distillation (ml) |
| V_3 | = | volume of absorbing solution (ml) used in the step 9.3.1 |

11. Alternative method

- Titrimetry with silver nitrate (APHA, 1989)

NB: * KCN is highly toxic. Avoid contact or inhalation.

AMMONIA (NITROGEN) $\mu\text{g l}^{-1}$

Spectrophotometric
Wavelength. 630 nm
Path length: 1 cm

Working Range. 10 - 500 $\mu\text{g l}^{-1}$ $\text{NH}_3\text{-N}$

Precision . at 200 $\mu\text{g l}^{-1}$ $\text{NH}_3\text{-N}$ 39.2%

Accuracy at 200 $\mu\text{g l}^{-1}$ $\text{NH}_3\text{-N}$ 2.4%

Interferences Alkalinity > 500 mg l^{-1} as CaCO_3
Acidity > 100 mg l^{-1} as CaCO_3

1. Application

1.1 The method is applicable to potable and fresh waters and effluents.

2. Summary of method

2.1 An intensely blue compound, indophenol is formed by the reaction of ammonia, with hypochlorite and phenol in the presence of a manganous salt as a catalyst.

3. Sampling

3.1 Direct, filtered through 0.45 μm membrane filter paper.

4. Preservation

4.1 Preserve samples with 0.8 ml conc. H_2SO_4 l^{-1} and store at 4 $^{\circ}\text{C}$.

5. Apparatus

5.1 UV/Visible spectrophotometer

5.2 Magnetic stirrer

6. Reagents and chemicals

6.1 Ammonia free water

6.2 Sodium hypochlorite solution (5%) [NaOCl]

6.3 Conc. hydrochloric acid [HCl]

6.4 Manganous sulphate [$\text{MnSO}_4 \cdot \text{H}_2\text{O}$]

6.5 Sodium hydroxide [NaOH]

- 6.6 Ammonium chloride [NH_4Cl]
- 6.7 Phenol [$\text{C}_6\text{H}_5\text{OH}$]
- 6.8 Conc. sulfuric acid [H_2SO_4]

7. Preparation of standards and reagents

- 7.1 Ammonia free distilled water
 - 7.1.1 Add 0.1 ml of conc. H_2SO_4 to 1 liter of deionized distilled water and re-distill.
 - 7.1.2 Discard the first 100 ml of the distillate and store the rest in an airtight bottle.
 - 7.1.3 Use ammonia free water for preparation of other reagents and for dilutions.
- 7.2 Hypochlorous acid reagent (prepare weekly)
 - 7.2.1 Add 10 ml 5% sodium hypochlorite solution (prepared from commercial bleach) to 40 ml ammonia free water (7.1).
 - 7.2.2 Adjust pH to 6.5 to 7.0 with dilute HCl solution.
- 7.3 Manganous sulphate solution (0.003 M)
 - 7.3.1 Dissolve 50 g of manganous sulphate in 100 ml ammonia free water.
- 7.4 Phenate reagent (prepare weekly)
 - 7.4.1 Dissolve 2.5 g NaOH and 10 g phenol in 100 ml ammonia free water.
- 7.5 Standard ammonium stock solution (1.00 ml \equiv 100 μg N \equiv 122 μg NH_3)
 - 7.5.1 Dissolve 381.9 mg anhydrous ammonium chloride and dilute to 1000 ml.
- 7.6 Working standard ammonium solution (1.00 ml \equiv 0.500 μg N \equiv 0.607 μg NH_3)
 - 7.6.1 Dilute 5.00 ml of the stock ammonium solution to 1000 ml with distilled water.

8. Calibration

- 8.1 Prepare a series of standards in the range of 0.1-5 μg $\text{NH}_3\text{-N}$ by diluting the working standard ammonium solution.
- 8.2 Measure the absorbance of the standards at 830 nm following the exact procedure given in 9.1 to 9.4.
- 8.3 Plot a calibration curve, absorbance versus $\text{NH}_3\text{-N}$ content.

KJELDHAL NITROGEN (ORGANIC NITROGEN) mg l^{-1}

Spectrophotometric
Wavelength. 425 nm
Path length 1 cm

Working Range: 4 - 40 mg l^{-1} N at 50 ml sample size

Precision . at 0.31 N mg l^{-1} 80%

Accuracy at 0.31 N mg l^{-1} +5 45%

Interferences: High nitrate concentrations

1. Application

- 1.1 The method is applicable for potable, fresh and saline waters and effluents.
- 1.2 The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia but may not convert the nitrogenous compounds of some industrial wastes.

2. Summary of method (Kjeldahl method)

- 2.1 The sample is digested in the presence of a mixture of catalysts and evaporated until SO_3 fumes are observed.
- 2.2 The residue is made alkaline and the ammonia is distilled to determine spectrophotometrically.

3. Sampling

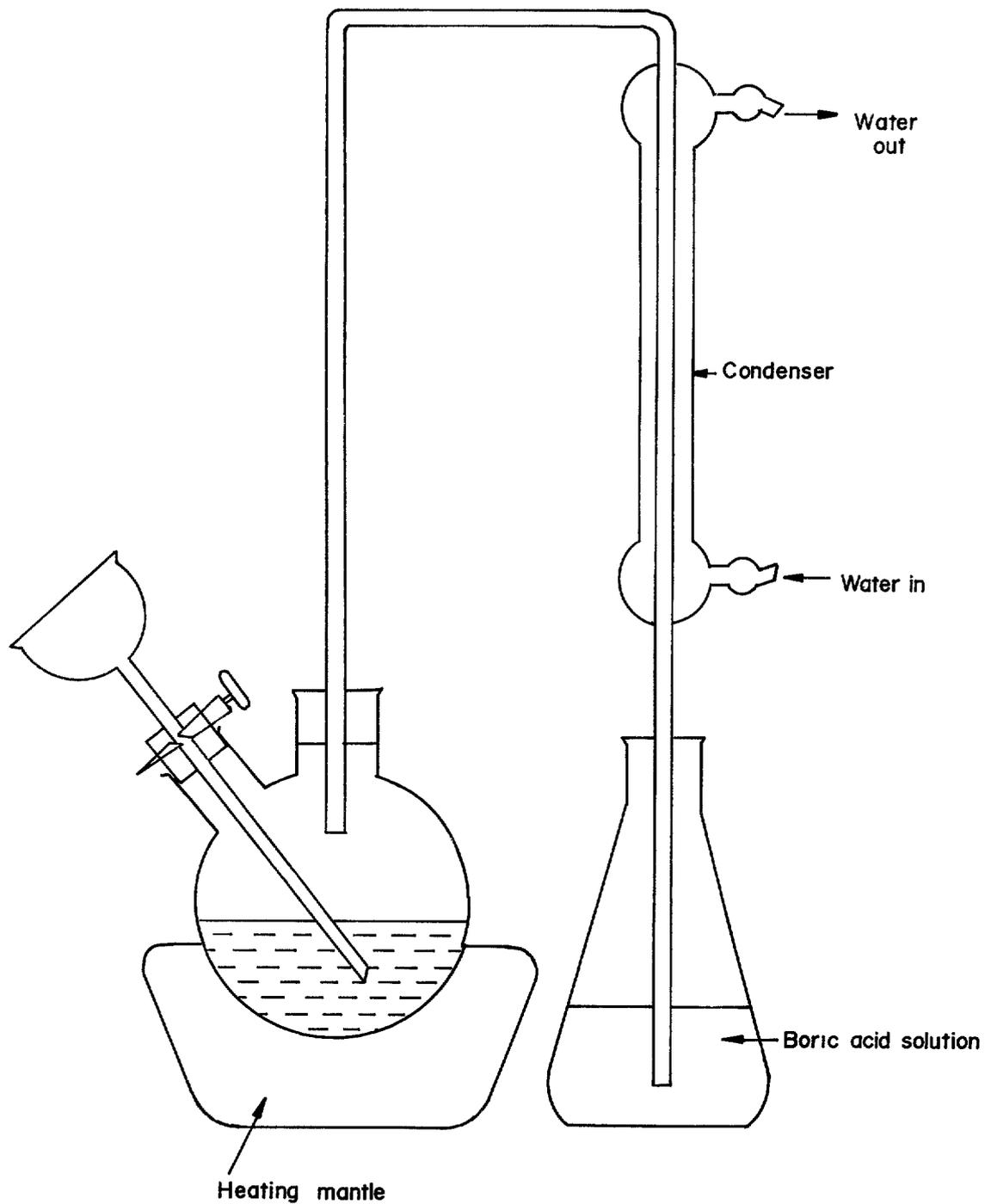
- 3.1 Direct, filtered through 0.45 μm membrane filter paper.

4. Preservation

- 4.1 Preserve samples by acidifying with H_2SO_4 to pH 1.5-2.0 and store at 4 $^{\circ}\text{C}$.

5. Apparatus

- 5.1 Digestion apparatus - A Kjeldahl flask (800 ml)
- 5.2 Distillation apparatus (Fig. 5.2)
- 5.3 UV/Visible spectrophotometer



Heating mantle

Fig. 5.2 Distillation apparatus

6. Reagents and chemicals

6.1 Ammonia free distilled water

- 6.2 Mercuric oxide [HgO]
- 6.3 Potassium sulphate [K₂SO₄]
- 6.4 Conc. sulfuric acid [H₂SO₄]
- 6.5 Sodium hydroxide [NaOH]
- 6.6 Sodium thiosulphate [Na₂S₂O₃·5H₂O]
- 6.7 Boric acid [H₃BO₃]
- 6.8 Ammonium chloride [NH₄Cl]
- 6.9 Nessler reagent or mercuric iodide and potassium iodide [HgI₂ and KI]

7 Preparation of standards and reagents

- 7.1 Ammonia free distilled water
 - 7.1.1 Add 0.1 ml of conc. H₂SO₄ to 1 liter of deionized distilled water and re-distill.
 - 7.1.2 Discard the first 100 ml of the distillate and store the rest in an airtight bottle.
 - 7.1.3 Use ammonia free water for preparation of other reagents and for dilutions.
- 7.2 Mercuric sulphate solution
 - 7.2.1 Dissolve 8 g red mercuric oxide in 50 ml of sulfuric (1+4) acid (10.0 ml conc. H₂SO₄ + 40.0 ml ammonia free water).
 - 7.2.2 Dilute to 100 ml with distilled water.
- 7.3 Sulfuric/mercuric sulphate/potassium sulphate solution
 - 7.3.1 Dissolve 133.5 g potassium sulphate in 850 ml ammonia free water and 200 ml conc. H₂SO₄.
 - 7.3.2 Add 25.0 ml mercuric sulphate (7.2) solution.
 - 7.3.3 Dilute to 1 liter with ammonia free water.
- 7.4 Sodium hydroxide/sodium thiosulphate solution
 - 7.4.1 Dissolve 500 g NaOH and 25 g sodium thiosulphate in ammonia free water.
 - 7.4.2 Dilute to 1 liter.
- 7.5 Boric acid solution
 - 7.5.1 Dissolve 20 g boric acid in ammonia free water.
 - 7.5.2 Dilute to 1 liter with ammonia free water.
- 7.6 Standard ammonium chloride stock solution (1000 mg l⁻¹ N)
 - 7.6.1 Dissolve 3.819 g ammonium chloride in ammonia free water.
 - 7.6.2 Make up to liter in a volumetric flask.
- 7.7 Working standard ammonium chloride solution (10 mg l⁻¹)
 - 7.7.1 Dilute 10.0 ml of the stock solution (7.6) with ammonia free water to 1 liter in a volumetric flask.
- 7.8 Sodium hydroxide solution (8 N)
 - 7.8.1 Dissolve 320 g of NaOH in ammonia free water and dilute to 1

liter.

7.9 Nessler reagent (commercially available, but manual preparation is preferred)

7.9.1 Dissolve 100 g of mercuric iodide and 70 g potassium iodide in a small volume of ammonia free water.

7.9.2 Add this mixture slowly to 500 ml of 8 N NaOH solution (7.8) with stirring and dilute to 1 liter.

8. Calibration

8.1 Measure aliquots of the working standard as given below into 50 ml volumetric flasks.

volume (ml) of working standard (10 mg ^l ⁻¹ NH ₃ -N)	mg NH ₃ -N per 50 ml
0.0	0.000
0.5	0.005
1.0	0.010
2.0	0.020
4.0	0.040
5.0	0.050
8.0	0.080
10.0	0.100

8.2 Dilute above aliquots of the working standard to 50 ml with ammonia free water to give 0 - 0.10 mg NH₃-N content, add 1 ml of Nessler reagent and mix well.

8.3 After 20 minutes, set zero absorbance at 425 nm with the blank.

8.4 Read the absorbance of the standards.

8.5 Plot the calibration curve of absorbance versus mg NH₃-N content of the standards.

9 Procedure

9.1 Macro-Kjeldahl system

9.1.1 Place a measured volume of the sample in an 800 ml Kjeldahl flask. The sample size can be determined from the following table:

Kjeldahl Nitrogen in sample (mg ^l ⁻¹)	Sample Size (ml)
4 - 40	50
8 - 80	25
20 - 200	10
40 - 400	05

- 9.1.2 Dilute the sample, if required, to 500 ml with ammonia free distilled water.
 - 9.1.3 Add 100 ml sulfuric acid/mercuric sulphate/potassium sulphate solution (7.3).
 - 9.1.4 Evaporate the mixture in the Kjeldahl apparatus until SO₃ fumes are observed and the solution turns colourless or pale yellow.
 - 9.1.5 Continue heating for 30 additional minutes, cool the residue and add 300 ml distilled water.
 - 9.1.6 Make the digestate alkaline by careful addition of 100 ml of sodium hydroxide/thiosulphate solution (7.4) without mixing.
 - 9.1.7 Connect the Kjeldahl flask immediately to the condenser with the tip of the condenser or an extension of the condenser tip below the level of the boric acid solution (7.5) in the receiving flask.
 - 9.1.8 Distill 300 ml at the rate of 8-10 ml min⁻¹ into 50 ml of 2% boric acid (7.5) contained in a 500 ml Erlenmeyer flask (receiving flask).
 - 9.1.9 Dilute the distillate to 500 ml in the flask.
- 9.2 Determination of ammonia in the distillate
- 9.2.1 Take an aliquot of the distillate and develop colour exactly in the same manner as the standards (8.2).
 - 9.2.2 Read the absorbance and calculate the Total Kjeldahl Nitrogen (TKN) concentration using the calibration curve.

10. Calculation

$$\text{Nitrogen (organic), mg l}^{-1} = TKN = \frac{A \times 1000}{V_3} \times \frac{V_1}{V_2}$$

where;

A	=	mg NH ₃ -N read from calibration curve.
V ₁	=	total volume of distillate after dilution in ml
V ₂	=	volume of distillate taken for nesslerization in ml (9.2.1)
V ₃	=	volume of original sample taken for distillation in ml

11. Alternative method

- Kjeldahl digestion followed by boric acid titration (APHA, 1989)

- NB:**
- * If high concentrations of nitrate interfere, use an anion exchange resin to remove the nitrate prior to the analysis.
 - * For concentrations above 1 mg l⁻¹ N, the titrimetric method is used for

determination of NH_3 .

- * For the range of 0.05 to 1400 mg l^{-1} N, potentiometric method may be used.
- * Do not use HgCl_2 for preservation because it will interfere with ammonia removal.

NITRITE (NITROGEN) mg l^{-1}

Spectrophotometric
Wavelength 543 nm
Path length: 1 cm

Working Range 0.01 - 1.0 mg l^{-1} NO_2^- - N

Precision at 0.04 mg l^{-1} ($\text{NO}_3^- + \text{NO}_2^-$) - N 12.5%
at 0.24 mg l^{-1} ($\text{NO}_3^- + \text{NO}_2^-$) - N 1.8%

Interferences: Strong oxidants (e.g. Fe(III)) or reductants (e.g. S^{2-})
Alkalinity > 800 mg l^{-1}

1. Application

1.1 The method is applicable to the determination of nitrite singly or nitrite and nitrate combined in potable, fresh and saline waters and effluents.

2. Summary of method

2.1 The diazonium compound is formed by diazotisation of sulfanilamide with nitrite in water under acidic conditions.

2.2 The diazo complex is coupled with N-(1-naphthyl)ethylenediamine dihydrochloride to produce a reddish-purple color which can be measured spectrophotometrically.

3. Sampling

3.1 Direct, filtered through 0.45 μm membrane filter paper.

4. Preservation

4.1 Store at 4 $^{\circ}\text{C}$ for 24-48 hours; **never use acid preservation.**

5. Apparatus

5.1 UV/Visible spectrophotometer

5.2 Volumetric flask

6. Reagents and chemicals

6.1 Sulfanilamide [$\text{NH}_2\text{C}_6\text{H}_4\text{SO}_2\text{NH}_2$]

6.2 N-(1-naphthyl) ethylene diamine dihydrochloride [$\text{C}_{12}\text{H}_{14}\text{N}_2 \cdot 2\text{HCl} \cdot \text{CH}_3\text{OH}$]

- 6.3 Sodium nitrite [NaNO₂] (analytical grade)
- 6.4 Conc. hydrochloric acid [HCl]

7. **Preparation of standards and reagents**

- 7.1 Buffer/colour developing reagent
 - 7.1.1 Dissolve 10 g sulfanilamide and 1 g N- (1-naphthyl) ethylene-diamine dihydrochloride in a mixture of 100 ml conc. H₃PO₄ and 800 ml of distilled water and dilute to 1 liter.
- 7.2 Standard nitrite stock solution (100 mg l⁻¹ NO₂⁻ - N)
 - 7.2.1 Dry several grams of anhydrous sodium nitrite in an oven for 3 hours at 80 °C and cool in a desiccator.
 - 7.2.2 Weigh 0.1493 g of dry sodium nitrite and dissolve in 1000 ml distilled water.
 - 7.2.3 Preserve with 2 ml chloroform per liter.
- 7.3 Working standard nitrite solution (1 mg l⁻¹ NO₂⁻ - N)
 - 7.3.1 Dilute 10.0 ml of the stock solution to 1000 ml with distilled water.
- 7.4 HCl (1+3) solution
 - 7.4.1 Add one volume of conc. HCl to 3 volumes of distilled water.

8. **Calibration**

- 8.1 Prepare a series of standards in 50 ml volumetric flasks as follows:

volume (ml) of working standard solution	concentration when diluted to 50 ml (mg l⁻¹ NO₂⁻ - N)
0.0	blank
0.5	0.01
1.0	0.02
1.5	0.03
2.0	0.04
3.0	0.06
4.0	0.08
5.0	0.10
10.0	0.20

- 8.2 Add 2 ml of buffer/colour developing reagent to each standard, mix well and allow colour to develop for at least 15 minutes. The reaction medium should be between pH 1.5 and 2.0 (check with a pH meter).
- 8.3 Read the absorbance by the spectrophotometer at 543 nm.

8.4 Plot the calibration curve, absorbance versus concentration of nitrite in mg l^{-1} .

9. Procedure

- 9.1 Adjust pH of the sample to 8 with HCl (1+3).
- 9.2 If necessary, filter the sample through a $0.45 \mu\text{m}$ pore size filter paper using the first 10 ml portion of the filtrate to rinse the filter flask.
- 9.3 Place 50 ml of the filtered sample or an aliquot diluted to 50 ml in a 50 ml volumetric flask.
- 9.4 Add 2 ml of buffer/colour developing reagent to the sample, mix and allow colour to develop at least for 15 minutes.
- 9.5 Read the absorbance at 543 nm.
- 9.6 Calculate NO_2^- concentration from the calibration curve.

10. Calculation

10.1

$$\text{NO}_2^- - \text{N}, \text{mg l}^{-1} = C \times D$$

where; C = concentration of $\text{NO}_2^- - \text{N}$ (mg l^{-1}) of the colour developed solution
D = dilution factor

10.2

$$\text{NO}_2^-, \text{mg l}^{-1} = 3.29 \times (\text{NO}_2^- - \text{N}) \text{mg l}^{-1}$$

NITRATE (NITROGEN) mg l^{-1}

Spectrophotometric
Wavelength - 543 nm
Path length - 1 cm

Working Range: 0.01 - 1.0 mg l^{-1} $\text{NO}_3\text{-N}$

Precision at 0.04 mg l^{-1} ($\text{NO}_3^- + \text{NO}_2^-$) 12.5%
at 0.24 mg l^{-1} ($\text{NO}_3^- + \text{NO}_2^-$) 1.8%

Interferences: High concentrations of metals

1. Application

- 1.1 The method is applicable to the determination of nitrite singly or nitrite and nitrate combined in potable, fresh and saline waters and effluents.
- 1.2 If only nitrate is desired, a separate determination must be made for nitrite and subsequent corrections made. The nitrite may be determined by the procedure without the reduction step.

2. Summary of method

- 2.1 Sample is passed through a column containing granulated copper-cadmium to reduce nitrate to nitrite.
- 2.2 The nitrite is determined by diazotising with sulfanilamide and coupling with N-(1-naphthyl) ethylenediamine dihydrochloride to form a highly colored azo dye which is measured spectrophotometrically.

3. Sampling

- 3.1 Direct, filtered through 0.45 μm membrane filter paper.

4. Preservation

- 4.1 Store at 4 $^{\circ}\text{C}$ for 24 hours, for longer preservation add 2 ml conc. H_2SO_4 per liter

5. Apparatus

- 5.1 Cu-Cd reduction column
- 5.2 UV/Visible spectrophotometer

6. Reagents and chemicals

- 6.1 Granulated Cd (40-80 mesh)
- 6.2 Copper sulphate [$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$]
- 6.3 Ammonium chloride [NH_4Cl]
- 6.4 Disodium ethylenediamine tetracetate [Na_2EDTA]
- 6.5 Sulfanilamide [$\text{NH}_2\text{C}_6\text{H}_4\text{SO}_2\text{NH}_2$]
- 6.6 N-(1-naphthyl) ethylenediamine dihydrochloride [$\text{C}_{12}\text{H}_{14}\text{N}_2 \cdot 2\text{HCl} \cdot \text{CH}_3\text{OH}$]
- 6.7 Conc. phosphoric acid [H_3PO_4]
- 6.8 Conc. ammonium hydroxide [NH_4OH]
- 6.9 Conc. hydrochloric acid [HCl]
- 6.10 Potassium nitrate [KNO_3] (analytical grade)
- 6.11 Potassium nitrite [KNO_2] (analytical grade)

7. Preparation of standards and reagents

- 7.1 Copper-cadmium colloid
 - 7.1.1 Wash cadmium granules with dilute HCl and rinse with distilled water. The colour of the granules should be silver.
 - 7.1.2 Swirl 25 g acid washed cadmium granules in 100 ml of 2% copper sulphate solution (7.6) for 5 minutes or until blue color partially fades, decant and repeat with fresh copper sulphate solution until a brown colloidal precipitate forms.
 - 7.1.3 Wash the copper-cadmium colloid with distilled water to remove all the precipitated copper. The color of Cd granules should be black.
- 7.2 Ammonium chloride / EDTA solution
 - 7.2.1 Dissolve 13 g ammonium chloride and 1.7 g disodium ethylenediamine tetraacetate in 900 ml of distilled water.
 - 7.2.2 Adjust pH to 8.5 with conc. ammonium hydroxide and dilute to 1 liter.
- 7.3 Dilute ammonium chloride/EDTA solution
 - 7.3.1 Dilute 300 ml of ammonium chloride/EDTA solution to 500 ml with distilled water.
- 7.4 Colour developing reagent
 - 7.4.1 Dissolve 10 g sulfanilamide and 1 g N-(1-naphthyl) ethylenediamine dihydrochloride in a mixture of 100 ml conc. H_3PO_4 and 800 ml of distilled water and dilute to 1 liter.
- 7.5 Hydrochloric acid (8 N)
 - 7.5.1 Dilute 50 ml of conc. HCl to 100 ml with distilled water.
- 7.6 Copper sulphate solution (2%)
 - 7.6.1 Dissolve 20 g of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 500 ml distilled water.

- 7.6.2 Dilute to 1 liter.
- 7.7 Standard nitrate stock solution (1000 mg^l⁻¹ NO₃⁻ - N)
- 7.7.1 Dissolve 7.218 g KNO₃ in distilled water and dilute to 1000 ml.
- 7.7.2 Preserve with 2 ml of chloroform per liter.
- 7.8 Standard nitrate working solution (10 mg^l⁻¹ NO₃⁻ - N)
- 7.8.1 Dilute 10.0 ml of standard nitrate stock solution (7.7) to 1000 ml with distilled water.
- 7.9 Standard nitrite stock solution (1000 mg^l⁻¹ NO₂⁻ - N)
- 7.9.1 Dissolve 6.072 g KNO₂ in 500 ml of distilled water.
- 7.9.2 Dilute to 1000 ml.
- 7.9.3 Preserve with 2 ml of chloroform and keep under refrigeration.
- 7.10 Standard nitrite working solution (10 mg^l⁻¹ NO₂⁻ - N)
- 7.10.1 Dilute 10.0 ml of the stock nitrite solution (7.9) to 1000 ml with distilled water.

8. Calibration

- 8.1 Prepare a series of standards by diluting the working standard solution in 100 ml volumetric flasks as follows:

Concentration (mg ^l ⁻¹) NO ₃ ⁻ - N	Volume (ml) of standard working solution added
0.00	0.0
0.05	0.5
0.10	1.0
0.20	2.0
0.50	5.0
1.00	10.0

- 8.2 Carry out the reduction of standards exactly as described for the samples (follow the steps 9.4-9.9). At least one nitrite standard should be compared to a reduced nitrate standard of the same concentration to verify the efficiency of the reduction column.
- 8.3 Obtain a standard curve by plotting the absorbance of standards against NO₃⁻ - N concentrations of standards (8.1).

9. Procedure

- 9.1 Turbidity removal of samples (usually not required for potable water)
- 9.1.1 Filter samples through a glass fiber filter or a 0.45 μm membrane filter paper.
- 9.2 Oil and grease removal of samples (usually not required for potable water)
- 9.2.1 Adjust pH of 100 ml of filtered sample to 2 by addition of conc. HCl in a separating funnel.

- 9.2.2 Add 25 ml of chloroform or carbon tetrachloride and shake well.
- 9.2.3 Discard the organic layer and repeat the step 9.2.2 with aqueous phase.
- 9.3 Preparation of reduction column
- 9.3.1 Use a glass column with a stopcock, 25 cm x 1 cm (25 ml burette may also be used).
- 9.3.2 Insert a glass wool plug to the bottom of the column and fill with distilled water.
- 9.3.3 Add sufficient Cu-Cd granules to produce a column, 18.5 cm long (maintain the water level above the granules).
- 9.3.4 Wash the column with 200 ml dilute NH₄Cl/EDTA solution (7.3).
- 9.3.5 Activate the column by passing 100 ml of a mixture of 25 ml of 1.0 mg l⁻¹ NO₃⁻ - N standard and 75 ml of NH₄Cl/EDTA solution (7.2) through the column.
- 9.4 If the pH of the sample is below 5 or above 9, adjust pH to between 5 and 9 with either conc. HCl or conc. NH₄OH.
- 9.5 Take 25 ml of sample or an aliquot diluted to 25 ml and add 75 ml of ammonium chloride/EDTA solution (7.2) and mix well.
- 9.6 Pour sample into the column and collect eluent at a rate of 7-10 ml min.⁻¹
- 9.7 Discard the first 25 ml, collect the rest of the sample (approximately 70 ml) in the original sample flask (samples, after passing through the column should not be allowed to stand longer than 15 minutes before addition of colour developing reagent).
- 9.8 Add 2 ml of colour developing reagent to 50 ml of sample collected in step 9.7. Allow 10 minutes for colour development and measure the absorbance at 543 nm within 2 hours.
- 9.9 Compute (NO₂⁻ + NO₃⁻) concentration of samples as nitrogen from the standard curve.
- 9.10 Follow the procedure for nitrite analysis and determine the nitrate concentration of the sample.

10. Calculation

10.1

$$(\text{NO}_2^- + \text{NO}_3^-) - \text{N}, \text{ mg l}^{-1} = \frac{C \times 25}{V}$$

where; C = concentration of nitrate from the standard curve as N
 V = volume of original sample taken in step 9.5

10.2 Calculation of NO_3^- - N concentration

$$\text{NO}_3^-, N \text{ mg l}^{-1} = [(\text{NO}_2^- + \text{NO}_3^-), N \text{ mg l}^{-1}] - [\text{NO}_2^-, N \text{ mg l}^{-1}]$$

10.3 Calculation of NO_3^- concentration

$$\text{NO}_3^- \text{ mg l}^{-1} \equiv 4.43 \times \text{NO}_3^-, N \text{ mg l}^{-1}$$

11. Alternative method

- Hydrazine reduction method (APHA, 1989)

PHOSPHORUS (AVAILABLE) mg l^{-1}

Spectrophotometric
Wavelength: 880 nm
Path length 20 mm

Working Range: 0.01 - 1.2 mg l^{-1} as P

Precision : at 0.029 mg l^{-1} P 34%
at 0.383 mg l^{-1} P 8%

Accuracy: at 0.029 mg l^{-1} P -4.95%
at 0.383 mg l^{-1} P -1.78%

Interferences: High concentrations of silica, salt concentrations > 20%

1. Application

1.1 The method is applicable to potable, fresh and saline waters and effluents.

2. Summary of method

2.1 Ammonium molybdate and antimony potassium tartrate react with orthophosphate to form an antimony phosphate molybdate complex.

2.2 The complex is reduced with ascorbic acid to form a deep blue molybdenum complex.

2.3 The colour intensity which is proportional to the phosphorous concentration is measured.

3. Sampling

3.1 Direct, filtered through 0.45 μm membrane filter paper.

4. Preservation

4.1 Store at -10°C after adding 40 mg HgCl_2 ml^{-1}

5. Apparatus

5.1 UV/Visible spectrophotometer

5.2 Acid-washed glassware (washed with HCl [1+3] followed by distilled water)

6. Reagents and chemicals

6.1 Phenolphthalein

- 6.2 Conc. sulfuric acid [H_2SO_4]
- 6.3 Ammonium molybdate [$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$]
- 6.4 Antimony potassium tartrate [$\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6\cdot \frac{1}{2}\text{H}_2\text{O}$]
- 6.5 Potassium dihydrogen phosphate [KH_2PO_4] (analytical grade)
- 6.6 Ascorbic acid [$\text{C}_6\text{H}_8\text{O}_6$]

7. Preparation of standards and reagents

- 7.1 Phenolphthalein indicator solution (5 g l^{-1})
 - 7.1.1 Dissolve 0.5 g of phenolphthalein in a mixture of 50 ml ethyl alcohol and 50 ml water.
- 7.2 Sulfuric acid (11 N)
 - 7.2.1 **Slowly** add 310 ml of concentrated sulfuric acid to about 600 ml of distilled water in a cool water bath.
 - 7.2.2 Cool the solution and dilute to 1 liter with distilled water.
- 7.3 Tartrate/molybdate solution
 - 7.3.1 Dissolve 0.13 g of antimony potassium tartrate in a one liter volumetric flask containing about 700 ml distilled water.
 - 7.3.2 Add 5.6 g of ammonium molybdate and shake the flask until dissolved.
 - 7.3.3 Cautiously add 70 ml of conc. H_2SO_4 acid while stirring.
 - 7.3.4 Cool the solution and dilute to 1 liter.
- 7.4 Combined reagent
 - 7.4.1 Dissolve 0.5 g of ascorbic acid in 100 ml of tartrate/molybdate solution (7.3).
- 7.5 Standard phosphorus solution ($1.00 \text{ ml} \equiv 0.0025 \text{ mg P}$)
 - 7.5.1 Dissolve 0.2197 g of potassium dihydrogen phosphate (dried at 105°C for 1 hour) in deionized distilled water and dilute to 1 liter.
 - 7.5.2 Prepare the standard phosphorus solution by diluting 50 ml of the stock solution to 1 liter with deionized distilled water.

8. Calibration

- 8.1 Prepare a series of standards covering the range of $0.01\text{-}1.2 \text{ mg l}^{-1}$ as P by diluting the standard phosphorous solution.
- 8.2 Treat the standards as given in the procedure (9.1-9.5).
- 8.3 Plot the calibration curve between phosphorous content and absorbance.

9. Procedure

- 9.1 Pipette 50 ml of the sample/standards or an aliquot diluted to 50 ml into a 125 ml Erlenmeyer flask.

PHOSPHOROUS (TOTAL) mg l^{-1}

Spectrophotometric
Wavelength - 880 nm
Path length - 20 mm

Working Range: 0.01 - 1.2 mg l^{-1} P

Precision at 0.110 mg l^{-1} P 30%
at 0.882 mg l^{-1} P 15%

Accuracy at 0.110 mg l^{-1} P +3.09%
at 0.882 mg l^{-1} P -0.92%

Interferences Arsenate and high concentrations of iron

1. Application

1.1 The method is applicable to potable, fresh and saline waters and effluents.

2. Summary of method

2.1 Phosphorous species are converted to orthophosphate by treating with ammonium persulphate with ammonium molybdate and antimony potassium tartrate in acid medium to form an antimony-phospho-molybdate complex. This complex is reduced to an intensely blue complex by ascorbic acid. The colour intensity is proportional to the phosphorus concentration.

3. Sampling

3.1 Direct, unfiltered

4. Preservation

4.1 Freeze or add 1 ml conc. HCl per litre; do not store in plastic bottles.

5. Apparatus

5.1 UV/Visible spectrophotometer

5.2 Acid washed glassware (washed with HCl [1+1] followed by distilled water).

5.3 Water bath, 95 °C

6. Reagents and chemicals

- 6.1 Phenolphthalein
- 6.2 Ammonium molybdate [(NH₄)₆Mo₇O₂₄·4H₂O]
- 6.3 Antimony potassium tartrate [K(SbO)C₄H₄O₆·½H₂O]
- 6.4 Ascorbic acid [C₆H₈O₆]
- 6.5 Conc. sulfuric acid [H₂SO₄]
- 6.6 Sodium bisulfite [NaHSO₃]
- 6.7 Ammonium persulphate [(NH₄)₂S₂O₈]
- 6.8 Potassium dihydrogen phosphate [KH₂PO₄] (analytical grade)

7. Preparation of standards and reagents

- 7.1 Ammonium molybdate-antimony potassium tartrate solution
 - 7.1.1 Dissolve 8 g of ammonium molybdate and 0.2 g antimony potassium tartrate in 800 ml of distilled water and dilute to 1 liter.
- 7.2 Ascorbic acid solution (prepare daily)
 - 7.2.1 Dissolve 60 g of ascorbic acid in 800 ml of distilled water and dilute to 1 liter.
 - 7.2.2 Add 2 ml of acetone.
- 7.3 Sulfuric acid (11 N)
 - 7.3.1 **Slowly add** 310 ml of conc. H₂SO₄ to approximately 800 ml distilled water in a cool water bath.
 - 7.3.2 Cool and dilute to 1000 ml.
- 7.4 Sulfuric acid (1 N)
 - 7.4.1 Dilute 90.9 ml of H₂SO₄ (11 N) to 1000 ml with distilled water.
- 7.5 Sodium bisulfite solution
 - 7.5.1 Dissolve 5.2 g of NaHSO₃ in 100 ml of H₂SO₄ (1 N).
- 7.6 Standard phosphorus stock solution (1.0 ml ≡ 0.1 mg P)
 - 7.6.1 Dissolve 0.4393 g of pre-dried (at 105 °C for one hour) potassium dihydrogen phosphate in deionized distilled water and dilute to 1 liter (add 2 ml conc. H₂SO₄).
- 7.7 Working standard phosphorus solution (1.0 ml ≡ 0.01 mg P)
 - 7.7.1 Dilute 100 ml of the stock phosphorus solution to 1 liter with deionized distilled water.

8. Calibration

- 8.1 Prepare a series of standards covering the range of 0.01-1.2 mg l⁻¹ P by diluting suitable volumes of working standard solution to 100 ml with distilled water.
- 8.2 Treat the standards as given in the procedure (9.1-9.7).

8.3 Plot the calibration curve, absorbance versus phosphorus concentration.

9. Procedure

- 9.1 Transfer 50 ml of sample or an aliquot diluted to 50 ml into a 125 ml Erlenmeyer flask.
- 9.2 Add 1 ml of 11 N sulfuric acid.
- 9.3 Add 0.4 g ammonium persulphate, mix and boil gently for approximately 30-40 minutes or until a final volume of about 10 ml is reached.
- 9.4 Cool and dilute to approximately 40 ml, filter and adjust the volume to 50 ml.
- 9.5 Add a drop of phenolphthalein indicator and adjust it to a faint pink by the addition of NaOH solution.
- 9.6 Add 4 ml of ammonium molybdate/antimony potassium tartrate reagent and mix well.
- 9.7 Add 2 ml of ascorbic acid solution and mix well.
- 9.8 After 5 minutes measure the absorbance of the sample at 880 nm with a spectrophotometer (the colour is stable for at least 1 hour).
- 9.9 Determine the phosphorus concentration from the calibration curve.

10. Calculation

$$\text{Total Phosphorous, } \text{mg l}^{-1} = \frac{C \times 50}{V}$$

where; C = phosphorus determined by the calibration curve, mg l^{-1}
V = volume of sample analyzed in ml

11. Alternative method

- Spectrophotometric method with stannous chloride (APHA, 1989)

NB:

- * If benthic deposits are present in the area being sampled, great care should be taken not to include these deposits.
- * For samples containing arsenate or high levels of iron, add 5 ml of sodium bisulfite, mix and place in a 95 °C water bath for 30 minutes. Cool and dilute to 50 ml.

SULPHATE mg l^{-1}

Nephelometric
Wavelength 420 nm

Working Range 1 - 40 mg l^{-1}

Detection limit. 1 $\text{mg l}^{-1} \text{SO}_4^{-2}$

Precision at 259 mg l^{-1} 4.7%

Accuracy: at 259 mg l^{-1} 1.9%

Interferences. Suspended matter, $\text{SiO}_2 > 500 \text{ mg l}^{-1}$

1. Application

1.1 The method is applicable to potable, fresh and saline waters and effluents.

2. Summary of method

2.1 Sulphate ion is reacted with BaCl_2 to form BaSO_4 suspension. The resulting turbidity is then determined by a nephelometer.

3. Sampling

3.1 Direct, filtered through 0.45 μm membrane filter paper.

4. Preservation

4.1 Store at 4 $^{\circ}\text{C}$

5. Apparatus

5.1 Magnetic stirrer

5.2 Nephelometer

5.3 Stop watch

5.4 Measuring spoon (capacity 0.2 to 0.3 ml)

6. Reagents and chemicals

6.1 Sodium sulphate [Na_2SO_4] (analytical grade)

6.2 Barium chloride [BaCl_2]

6.3 Sodium carbonate [Na_2CO_3]

6.4 Sodium chloride [NaCl]

6.5 Conc. hydrochloric acid [HCl]

6.6 Ethyl alcohol [$\text{C}_2\text{H}_5\text{OH}$]

6.7 Glycerol [C₃H₈O₃]

7. Preparation of standards and reagents

7.1 Conditioning reagent

7.1.1 Add 30 ml conc. HCl, 100 ml 95 % ethanol and 75 g NaCl to 300 ml distilled water.

7.1.2 Add 50 ml glycerol and mix well.

7.2 Standard sulphate stock solution (1.00 ml \equiv 0.100 mg SO₄²⁻)

7.2.1 Dissolve 147.9 mg anhydrous Na₂SO₄ (dried at 105 °C for 1 hour) in deionized distilled water in a one liter volumetric flask.

7.2.2 Dilute to the mark.

8. Calibration

8.1 Prepare a series of standards covering the range of 1-40 mg l⁻¹ SO₄²⁻ by diluting the standard sulphate stock solution.

8.2 Treat the standards as given in the procedure (9.1-9.3).

8.3 Plot a calibration curve between the sulphate concentration and nephelometric reading.

9. Procedure

9.1 Formation of barium sulphate suspension

9.1.1 Add 100 ml sample or standard (or a suitable portion diluted to 100 ml) into a 250 Erlenmeyer flask.

9.1.2 Add exactly 5 ml conditioning reagent and stir well.

9.1.3 While the solution is being stirred, add a measuring spoonful of BaCl₂ crystals and start timing immediately.

9.1.4 Stir exactly for 1 minute at a constant speed.

9.2 Measurement of sulphate content

9.2.1 Immediately after the stirring period has ended, pour solution (sample or standard) into an absorbance cell.

9.2.2 Measure turbidity at 30 second intervals for 4 minutes at 420 nm.

9.2.3 Record the maximum reading obtained in the 4 minute period.

9.3 Correction for sample colour and turbidity

9.3.1 Run a sample blank using the procedure 9.1 and 9.2 without the addition of barium chloride.

9.3.2 Subtract the sample blank reading from the BaCl₂ added sample reading.

9.4 Read the sulphate content of the sample from the calibration curve.

10. **Calculation**

$$SO_4^{2-} \text{ mg l}^{-1} = 100 \times \frac{C}{V}$$

where; C = SO_4^{2-} concentration corrected for colour and turbidity (from the calibration curve)
V = volume of the original sample (ml)

11. **Alternative method**

- Spectrophotometric method with thymol blue (USEPA, 1983)

Working Range 0.5 - 25 mg l^{-1}	Detection limit 0.5 mg l^{-1}
Interferences	Oxidizing and reducing agents, nitrate ions, organic matter and $\text{Fe} > 1 \text{ mg l}^{-1}$ The azide modification removes interference caused by nitrite, permanganate modification for the presence of ferrous ion, alum flocculation modification for the presence of suspended solids and the copper sulphate/sulfamic acid flocculation modification for activated-sludge mixed liquor

1. Application

1.1 The method is applicable to potable, fresh and saline waters and effluents.

2. Summary of method

2.1 Oxygen in the water sample is reduced by divalent manganous ion in the presence of alkali forming higher valance state manganic ion.

2.2 Upon acidification with sulfuric acid, the manganic ion is reverted to divalent state releasing iodine from the potassium iodide in the solution.

2.3 The iodine is then titrated with sodium thiosulphate and the dissolved oxygen concentration of the sample is calculated from the strength and the volume of the titrant.

3. Sampling

3.1 Direct, unfiltered

3.2 Fill the bottle very carefully without trapping air bubbles.

4. Apparatus

4.1 BOD incubation bottles with tapered ground glass pointed stoppers and flared mouths (capacity 300 ml)

4.2 Graduated pipettes (2.0 ml)

5. Reagents and chemicals

5.1 Manganous sulphate [$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$]

5.2 Sodium hydroxide [NaOH]

- 5.3 Sodium iodide [NaI]
- 5.4 Sodium azide [NaN₃]
- 5.5 Salicylic acid [C₆H₄(OH)COOH]
- 5.6 Chloroform [CHCl₃]
- 5.7 Conc. sulfuric acid [H₂SO₄]
- 5.8 Starch
- 5.9 Potassium fluoride [KF.2H₂O]
- 5.10 Sodium thiosulphate [Na₂S₂O₃.5H₂O] (analytical grade)
- 5.11 Potassium bi-iodate [KH(IO₃)₂]

6. Preparation of standards and reagents

- 6.1 Manganous sulphate solution
 - 6.1.1 Dissolve 480 g Manganous sulphate in distilled water and dilute to one liter.
- 6.2 Alkaline iodide/azide solution
 - 6.2.1 Dissolve 500 g of sodium hydroxide and 135 g of NaI in distilled water and dilute to one liter (or 700 g of KOH + 150 g of KI).
 - 6.2.2 Add 10 g of sodium azide dissolved in 40 ml of distilled water.
- 6.3 Starch solution
 - 6.3.1 Prepare an emulsion of 10 g soluble starch and 0.2 g salicylic acid in a beaker with a small quantity of distilled water.
 - 6.3.2 Pour this emulsion into one liter of boiling water and allow to boil for few minutes.
 - 6.3.3 Let settle overnight.
 - 6.3.4 Store the clear supernatant in an airtight bottle.
- 6.4 Potassium fluoride solution
 - 6.4.1 Dissolve 40 g potassium fluoride in distilled water.
 - 6.4.2 Dilute to 100 ml.
- 6.5 Standard sodium thiosulphate (0.0375 N) solution (1 ml is equivalent to 0.300 mg of DO)
 - 6.5.1 Dissolve 9.3075 g of sodium thiosulfate in distilled water and dilute to 1 liter.
 - 6.5.2 Preserve by adding 5 ml of chloroform.
 - 6.5.3 Standardize with 0.0375 N potassium bi-iodate solution procedure (7.1).
- 6.6 Standard potassium bi-iodate (0.0375 N) solution
 - 6.6.1 Dissolve 4.873 g of potassium bi-iodate (dried for 2 hours at 103 °C) in 1000 ml distilled water.

7. Procedure

- 7.1 Standardization of sodium thiosulphate
 - 7.1.1 Dissolve approximately 2 g of potassium iodide in 100 to 150 ml distilled water.
 - 7.1.2 Add 10 ml of 10% H₂SO₄ followed by 20 ml standard potassium bi-iodate solution (6.6).
 - 7.1.3 Place in the dark for 5 minutes.
 - 7.1.4 Dilute to 300 ml with distilled water and titrate with the sodium thiosulphate solution (6.5) to a pale straw colour.
 - 7.1.5 Add 1-2 ml starch solution and continue the titration carefully until the blue color disappears.
 - 7.1.6 Repeat the titration at least twice and calculate the normality of sodium thiosulphate
- 7.2 To the sample collected in the BOD incubation bottle (300 ml), **immediately** add 2 ml of the manganous sulphate solution.
- 7.3 Add 2 ml of the alkaline iodide-azide solution.
- 7.4 Stopper with care to exclude air bubbles and mix well by inverting the bottles several times.
- 7.5 When the precipitate is settled, shake again.
- 7.6 When settling has produced a precipitate less than 1/3 of the bottle volume, transfer the sample bottle to the laboratory, fully dipped in a water bath at ambient temperature.
- 7.7 In the laboratory, add 2 ml of conc. H₂SO₄ by allowing the acid to run down the neck of the bottle.
- 7.8 Re-stopper and mix by gentle inversion until the iodine is uniformly distributed throughout the bottle.
- 7.9 Transfer the entire bottle content by inversion into a 500 ml wide mouth flask.
- 7.10 Titrate with 0.0375 N thiosulphate solution to pale straw colour.
- 7.11 Add 1-2 ml of starch solution and continue the titration to the first disappearance of the blue color.

8. Calculation

- 8.1 Each ml of 0.0375 N sodium thiosulphate titrant is equivalent to 1 mg DO when the entire bottle content is titrated.
- 8.2 The solubility of DO in distilled water,

$$DO, \text{ mg l}^{-1} = \frac{(P - \mu) \times 0.878}{(35 + T) 0.698} \quad \text{at } 0 - 30^{\circ}\text{C}$$

$$DO, \text{ mg l}^{-1} = \frac{(P - \mu) \times 0.827}{(49 + T) 0.698} \quad \text{at } 30-50^{\circ}\text{C}$$

where; P (mmHg) = barometric pressure
 T ($^{\circ}$ C) = temperature
 μ (mmHg) = saturated vapor pressure

8.3 If the results are desired in ml^{-1} DO, multiply mg l^{-1} DO by 0.698.

9. **Alternative method**

- Oxygen membrane electrode method (Fresenius, 1988)

NB: * If ferric ion is present (100 to 200 mg l^{-1}), add 1.0 ml of KF solution before acidification.
 * Add starch after getting the pale straw colour.
 * Conduct the titration on a magnetic stirrer to get a sharp end point.

Working Range 0.5 - 25 mg l^{-1} **Detection limit** 0.5 mg l^{-1} DO**Precision** at 2.1 mg l^{-1} BOD_5 , 33%**1. Application**

1.1 The method is applicable to potable, fresh and saline waters and effluents.

2. Summary of method

2.1 Dissolved oxygen is measured initially in the air saturated sample and after incubation for 5 days.

2.2 BOD_5 is calculated from the difference between dissolved oxygen before and after incubation.

3. Sampling

3.1 Direct, unfiltered

3.2 Fill the bottle very carefully without trapping air bubbles.

4. Apparatus

4.1 Standard BOD bottles (300 ml capacity)

4.2 Water bath (thermostatically controlled at 25 ± 1 °C)

5. Reagents and chemicals

5.1 Potassium dihydrogen phosphate [KH_2PO_4]

5.2 Potassium hydrogen phosphate [K_2HPO_4]

5.3 Sodium hydrogen phosphate [$\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$]

5.4 Magnesium sulphate [$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$]

5.5 Calcium chloride [CaCl_2]

5.6 Ferric chloride [$\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$]

5.7 Sodium sulfite [Na_2SO_3]

5.8 Ammonium chloride [NH_4Cl]

5.9 Conc. sulfuric acid [H_2SO_4]

5.10 Sodium hydroxide [NaOH]

5.11 2-chloro-8-(trichloromethyl) pyridine

6. Preparation of standards and reagents

6.1 Phosphate buffer solution

6.1.1 Dissolve 8.5 g potassium dihydrogen phosphate, 21.75 g potassium hydrogen phosphate, 33.4 g sodium hydrogen phosphate and 1.7 g ammonium chloride in about 500 ml distilled water and dilute to 1 liter (pH should be 7.2 without adjustment.)

6.2 Magnesium sulphate solution

6.2.1 Dissolve 22.5 g magnesium sulphate in distilled water.

6.2.2 Dilute to 1 liter.

6.3 Calcium chloride solution

6.3.1 Dissolve 27.5 g calcium chloride in distilled water.

6.3.2 Dilute to 1 liter.

6.4 Ferric chloride solution

6.4.1 Dissolve 0.25 g ferric chloride in distilled water.

6.4.2 Dilute to 1 liter.

6.5 Acid solution

6.5.1 Add 28 ml conc. H_2SO_4 acid **slowly** to distilled water and dilute to 1 liter in a cool water bath.

6.6 Alkali solution

6.6.1 Dissolve 40 g sodium hydroxide in distilled water and dilute to 1 liter.

6.7 Sodium sulfite solution (prepare daily)

6.7.1 Dissolve 1.575 g of sodium sulfite in 1000 ml distilled water.

6.8 Ammonium chloride solution

6.8.1 Dissolve 1.15 g ammonium chloride in about 500 ml distilled water and adjust pH to 7.2 with NaOH solution.

6.8.2 Dilute to 1 liter.

6.9 Preparation of dilution water

6.9.1 Place a desired volume of distilled water in a suitable bottle and add 1 ml each of phosphate buffer, $MgSO_4$, $CaCl_2$ and $FeCl_3$ solutions per liter of water (if desired, seed dilution water by adding a small amount of micro-organisms e.g. untreated waste water).

6.9.2 Before use, bring temperature of dilution water to 25 °C.

6.9.3 Saturate with Dissolved Oxygen (DO) by shaking in a partially filled bottle or by aerating with organic free filtered air.

6.9.4 Store in cotton plugged bottles long enough for water to become saturated with DO.

7. Procedure

7.1 Sample pretreatment

- 7.1.1 Neutralize samples to pH 6.5 to 7.5 with a maximum volume of 1.5 ml of either sulfuric acid or sodium hydroxide.
- 7.1.2 If residual chlorine is present, dechlorinate the sample by adding Na_2S .
- 7.1.3 For samples containing more than 9 mg l^{-1} DO at 25°C , to prevent loss of oxygen during incubation, reduce DO to saturation level by bringing the sample to about 20°C in a partially filled bottle while agitating by vigorous shaking.
- 7.1.4 In case of BOD determination of waste water, add 3 mg of 2-chloro-8 (trichloro methyl) pyridine to each 300 ml bottle before capping or add sufficient amounts to the dilution water to make a final concentration of 10 mg l^{-1}

7.2 Dilution technique

- 7.2.1 Dilute different waste water types in BOD bottles as given below with dilution water.

Type of water	Dilution
Industrial waste	0-1%
Raw and settled waste water	1-5%
Biologically treated effluent	5-25%
Polluted river water	25-100%

- 7.2.2 When seeding is necessary, add seed directly to dilution water or directly to the BOD bottles.
- 7.2.3 Fill bottles with enough dilution water, so that insertion of stopper will displace all air, leaving no bubbles. Be careful not to spill the sample.
- 7.2.4 Incubate tightly stoppered BOD bottles covered with aluminium foil at $25 \pm 1^\circ\text{C}$ dipped in a water bath for 5 days.

7.3 Determination of initial DO

- 7.3.1 To the original sample (300 ml) collected in the BOD incubation bottle or the sample diluted to 300 ml with dilution water, **immediately** add 2 ml of the manganous sulphate solution.
- 7.3.2 Add 2 ml of the alkaline iodide-azide solution.
- 7.3.3 Stopper, with care to exclude air bubbles and mix well by inverting the bottles several times.
- 7.3.4 When the precipitate is settled, shake again.
- 7.3.5 When settling has produced a precipitate less than 1/3 of the bottle volume, transfer the sample bottle to the laboratory, fully dipped in a water bath at ambient temperature.
- 7.3.6 In the laboratory, add 2 ml of conc. H_2SO_4 by allowing the acid to

- run down the neck of the bottle.
- 7.3.7 Re-stopper and mix by gentle inversion until the iodine is uniformly distributed throughout the bottle.
- 7.3.8 Transfer the entire bottle content by inversion into a 500 ml wide mouth flask.
- 7.3.9 Titrate with 0.0375 N thiosulphate solution to pale straw color, add 1-2 ml of starch solution and continue the titration to the first disappearance of the blue colour.
- 7.4 Determination of final DO
- 7.4.1 Follow the procedure 7.3.1-7.3.9 for the sample incubated for 5 days.

8. Calculation

- 8.1 Each ml of 0.0375 N sodium thiosulphate titrant is equivalent to 1mg DO when the entire bottle content is titrated.
- 8.2 When dilution water is not seeded:

$$BOD_5 \text{ mg l}^{-1} = (D_1 - D_2) \times D$$

- 8.3 When dilution water is seeded:

$$BOD_5 \text{ mg l}^{-1} = [(D_1 - D_2) - (B_1 - B_2) f] \times D$$

- where:
- | | | |
|-------|---|--|
| D_1 | = | DO of diluted sample immediately after preparation (mg l ⁻¹) |
| D_2 | = | DO of diluted sample after 5 day incubation at 25 °C (mg l ⁻¹) |
| D | = | dilution factor |
| B_1 | = | DO of seed control before incubation (mg l ⁻¹) |
| B_2 | = | DO of seed control after incubation (mg l ⁻¹) |
| f | = | (% seed in diluted sample)/(% seed in seed control) |

9. Alternative method

- Oxygen membrane electrode method (Fresenius, 1988)

- NB:**
- * It is necessary to have micro-organisms capable of oxidizing the biodegradable organic matter in the samples that do not contain sufficient microbial populations.
 - * Long storage between collection and analysis may degrade the samples,

resulting in low BOD values.

- * Special study and treatment are needed for the samples containing toxic substances such as industrial wastes.
- * Dilution that results in a range of 1 to 2 mg/l⁻¹ of residual DO after 5 days of incubation produces the most reliable results.

Working Range. 5 - 50 mg^l⁻¹ COD

Precision : at 12.3 mg^l⁻¹ COD 34%

Accuracy at 12.3 mg^l⁻¹ COD 0.3%

Interferences Traces of organic matter and chlorides

1. **Application**

1.1 The method is applicable to potable, fresh and saline waters and effluents.

2. **Summary of method**

2.1 Organic and oxidizable inorganic substances in the sample are oxidized by potassium dichromate in acid medium.

2.2 The excess dichromate is titrated with standard ferrous ammonium sulphate.

3. **Sampling**

3.1 Direct, filtered through 0.45 μm membrane filter paper.

4. **Preservation**

4.1 Preserve at 4 °C with H₂SO₄ to pH < 2

5. **Apparatus**

5.1 Reflux apparatus

The apparatus should consist of a 500 ml Erlenmeyer flask or a 300 ml round bottom flask made of heat-resistant glass connected to a 12 inch Allihn condenser by means of a ground glass joint.

6. **Reagents and chemicals**

6.1 Distilled water (should be very low in organic matter)

6.2 Potassium dichromate [K₂Cr₂O₇] (analytical grade)

6.3 Conc. sulfuric acid [H₂SO₄]

6.4 Ferrous ammonium sulphate [Fe(NH₄)₂(SO₄)₂.6H₂O]

- 6.5 Ferrous sulphate [$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$]
- 6.6 Mercuric sulphate [HgSO_4]
- 6.7 Potassium hydrogen phthalate [$\text{C}_8\text{H}_5\text{O}_4\text{K}$]
- 6.8 1-10 (ortho) Phenanthroline
- 6.9 Silver sulphate [Ag_2SO_4]

7. Preparation of standards and reagents

- 7.1 Organic free distilled water
 - 7.1.1 Prepare organic free water by treating distilled water with activated carbon followed by mixed-bed ionization and filtration through membrane filter paper.
 - 7.1.2 Irradiate under ultra violet light for one hour and purge nitrogen just before use.
- 7.2 Standard potassium dichromate solution (0.25 N)
 - 7.2.1 Dissolve 12.259 g potassium dichromate (dried at 103°C for two hours) in distilled water (organic free).
 - 7.2.2 Dilute to 1000 ml.
- 7.3 Sulfuric acid/silver sulphate solution
 - 7.3.1 Add well ground 5.04 g of silver sulphate in 500 ml of conc. H_2SO_4 . Keep for two days to dissolve Ag_2SO_4 .
- 7.4 Standard ferrous ammonium sulphate solution (0.25 N)
 - 7.4.1 Dissolve 98 g of ferrous ammonium sulphate in distilled water (organic free).
 - 7.4.2 Add 20 ml conc. H_2SO_4 , cool and dilute to 1 liter (standardize this solution daily against $\text{K}_2\text{Cr}_2\text{O}_7$ solution.)
- 7.5 Phenanthroline/ferrous sulphate indicator solution (Ferrioin indicator)
 - 7.5.1 Dissolve 1.48 g of 1-10 (ortho) phenanthroline monohydrate, together with 0.70 g of ferrous sulphate in 100 ml of distilled water (organic free).

8. Procedure

- 8.1 Standardization of ferrous ammonium sulphate solution
 - 8.1.1 Add 25.0 ml of 0.25 N potassium dichromate solution to 200 ml of distilled water (organic free).
 - 8.1.2 Add 20 ml of conc. H_2SO_4 carefully and cool.
 - 8.1.3 Add three drops of ferrioin indicator and titrate with ferrous ammonium sulphate solution (7.4) to a sharp end point from blue green to reddish brown.
 - 8.1.4 Calculate the normality of ferrous ammonium sulphate solution using the formula given below:

$$\text{Normality} = \frac{(V_1) (0.25)}{V_2}$$

where; V_1 = volume of $K_2Cr_2O_7$ (ml)
 V_2 = volume of $Fe(NH_4)_2(SO_4)_2$ (ml)

- 8.2 Transfer 50 ml of sample (or an aliquot diluted to 50 ml) into a reflux flask with few glass beads and 1 g of $HgSO_4$
- 8.3 Add very slowly 5.0 ml conc. H_2SO_4 while mixing to dissolve mercuric sulphate (cool while mixing to avoid possible loss of volatile materials).
- 8.4 Place reflux flask in an ice bath and slowly add (with swirling) 25 ml of 0.25 N $K_2Cr_2O_7$
- 8.5 Connect the flask to the condenser and turn on the water cooler.
- 8.6 Add 70 ml of sulfuric acid/silver sulphate solution (7.3) through the open end of the condenser and mix well before heating.
- 8.7 Cover the open end of the condenser with a small beaker.
- 8.8 Apply heat to the flask and reflux for 2 hours.
- 8.9 Allow the flask to cool and wash down the condenser with about 25 ml of distilled water (organic free).
- 8.10 Transfer the solution volumetrically to a 500 ml Erlenmeyer flask.
- 8.11 Dilute the solution to about 300 ml with distilled water and allow the solution to cool to room temperature.
- 8.12 Add 8 to 10 drops of ferroin indicator to the solution and titrate the excess dichromate with 0.25 N ferrous ammonium sulphate solution (colour change at the end point-a blue-green to a reddish brown).
- 8.13 Simultaneously run a blank determination using organic free distilled water in place of the sample.

9. Calculation

$$COD, \text{ mg l}^{-1} = \frac{(V_1 - V_2) N \times 8000}{V_3}$$

where; V_1 = volume of $Fe(NH_4)_2(SO_4)_2$ solution required for titration of the blank (ml)
 V_2 = volume of $Fe(NH_4)_2(SO_4)_2$ solution required for titration of the sample (ml)
 N = normality of the $Fe(NH_4)_2(SO_4)_2$ solution ($eq l^{-1}$)
 V_3 = volume of original sample used for titration (ml)

NB: * When chloride in the sample exceeds 1000 mg l⁻¹, add mercuric sulphate to the digestion flask to complex the chlorides, thereby eliminating the interference.

Working Range 0.5 - 40 mg l ⁻¹ C	Detection limit 0.1 mg l ⁻¹ C
Precision at 2.2 mg l ⁻¹ C 5.9% at 38.0 mg l ⁻¹ C 3.7%	Accuracy at 2.2 mg l ⁻¹ C 5.9% at 38.0 mg l ⁻¹ C 3.7%
Interferences. Chloride > 0.1%; excessive acidification	

1. Application

1.1 The method is applicable to potable, fresh and saline waters and effluents.

2. Summary of method

2.1 The sample is acidified to remove inorganic carbon and oxidized with persulphate.

2.2 The resultant CO₂ is measured by a **Total Organic Carbon Analyzer**.

3. Sampling

3.1 Direct, filtered through 0.45 μm membrane filter paper.

4. Preservation

4.1 Preserve at 4 °C with H₂SO₄ to pH < 2

5. Apparatus

5.1 Total Organic Carbon Analyzer

6. Reagents and chemicals

6.1 Distilled water (organics free)

6.2 Conc. sulfuric acid [H₂SO₄]

6.3 Potassium biphthalate [C₈H₅O₄K] (analytical grade)

6.4 Sodium carbonate [Na₂CO₃] (analytical grade)

6.5 Sodium bicarbonate [NaHCO₃] (analytical grade)

6.6 Potassium persulphate [K₂S₂O₈]

6.7 Phosphoric acid [H₃PO₄] (85%)

- 6.8 Air (CO₂ free)
- 6.9 Any gas (CO₂ free)

7. Preparation of standards and reagents

- 7.1 Organic free water
 - 7.1.1 Prepare organic free water by treating with activated carbon followed by mixed-bed ionization and filtration through membrane filter paper.
 - 7.1.2 Irradiate under ultra violet light for one hour and purge with nitrogen just before use.
- 7.2 Standard organic carbon stock solution (1 ml ≡ 1 mg C)
 - 7.2.1 Dissolve 2.1254 g anhydrous potassium biphthalate in organic free water and dilute to 1000 ml.
 - 7.2.2 Preserve by acidifying with H₂SO₄ to pH < 2.
- 7.3 Standard inorganic carbon stock solution (1 ml ≡ 1 mg C)
 - 7.3.1 Dissolve 4.4122 g anhydrous sodium carbonate (dried at 105 °C for 1 hour) in organic free distilled water.
 - 7.3.2 Add 3.497 g anhydrous sodium bicarbonate and dilute to 1000 ml.
 - 7.3.3 Do not acidify (keep tightly stoppered).
- 7.4 Phosphoric acid solution (1.2 N)
 - 7.4.1 Add 83 ml H₃PO₄ (85%) to organic free distilled water and dilute to 1 liter.

8 Calibration

- 8.1 Prepare two series of standards covering the range of 0.5 - 40 mg l⁻¹ C by diluting both organic and inorganic standard stock solutions.
- 8.2 Inject the standards and calibrate the instrument according to the instruction manual (use organic free acidified distilled water as blank).
- 8.3 Plot the calibration curve of peak area of each standard versus concentration of organic and inorganic carbon (mg l⁻¹) respectively.

9. Procedure

- 9.1 If inorganic carbon is to be measured, inject samples directly and determine sample concentrations from the calibration curve for inorganic carbon.
- 9.2 If inorganic carbon is to be removed, transfer a portion of 10-15 ml of the sample to a 30 ml beaker and add phosphoric acid to reduce pH to 2 or less. Purge with gas for 10 minutes and then inject samples to the total carbon analyzer to determine organic carbon concentration of the samples

from the calibration curve for organic carbon.

10. **Calculation**

10.1 Read non-purgeable (i.e. non volatile) organic carbon concentration from the calibration curve.

11. **Alternative method**

- UV promoted persulphate oxidation method (USEPA, 1983)

NB: * Chloride interference can be eliminated by the addition of mercuric nitrate to the persulphate solution.

5.5 Microbiological Parameters

TOTAL COLONY COUNT Colonies 1 ml⁻¹

Pour Plate Culture

1. Application

1.1 The method is applicable to potable and surface water and effluents.

2. Definition

2.1 In the context of the method, the colonies are comprised of bacteria yeast and moulds capable of growing under specific conditions.

3. Summary of method

3.1 After mixing of test volumes of water samples with molten Yeast-Extract Agar in Petri dishes, incubate under the conditions specified and count the colonies that are developed.

4. Apparatus

- 4.1 Incubator with a thermostat
- 4.2 Petri dishes (10 cm diameter)
- 4.3 Conical flasks
- 4.4 Thermometer (precision 0.1 °C)
- 4.5 Pipettes (bacteriological)
- 4.6 Gas burner (or spirit lamp)
- 4.7 Colony counter
- 4.8 Water bath
- 4.9 Autoclave

5. Reagents and culture media

- 5.1 Potassium mono hydrogen phosphate [K₂HPO₄]
- 5.2 Sodium hydroxide [NaOH]
- 5.3 Magnesium chloride [MgCl₂]
- 5.4 **Nutrient Agar** (pH = 7.0 ± 1 after sterilization) prepared by mixing
 - Tryptone 5.0 g
 - Yeast extract 2.5 g
 - Glucose 1.0 g
 - Agar 15.0 g

Sterilized distilled water 1 l

6. Procedure

6.1 Preparation of samples

6.1.1 Invert the sample bottle rapidly several times in order to disperse any sediment.

6.1.2 Remove the stopper or cap, and retain in the hand.

6.1.3 Sterile the mouth of the bottle with a flame, pour off some of the contents, replace the stopper or cap and again shake the bottle in order to distribute any organisms uniformly throughout the water.

6.2 Diluent (quarter-strength **Ringer's Solution**)

6.2.1 **Ringer's Solution** (quarter-strength)

Sodium chloride 2.250 g

Potassium chloride 0.105 g

Calcium chloride (anhydrous) 0.120 g

Sodium bicarbonate 0.050 g

Distilled water 1 l

Dissolve the ingredients and disperse them in convenient values. Sterile by autoclaving at 121 °C for 15 minutes.

6.3 Making the dilution

6.3.1 Measure out 90 or 9 ml of the diluent into sterile dilution bottles or tubes.

6.3.2 Make one or more ten fold dilutions by transferring one volume of water sample to 9 volumes of diluent and so on.

6.3.3 Prepared sufficient amount for each dilution for all the tests to be carried out on the sample.

6.4 Preparation of the sample

6.4.1 Use the raw sample, make one, two or more ten fold dilutions of it according to the bacteria content expected.

6.4.2 Measure 1 ml volumes from the highest dilution into each of the two Petri dishes and, using the same pipette, from the other dilutions and finally from the undiluted sample into each of the four separate Petri dishes.

6.4.3 Pour 15 ml of Yeast-Extract Agar, (previously melted and cool to 45-50 °C) into each Petri dish.

6.4.4 Immediately mix the water and the agar by rapid but gentle to-and-fro and circular movements for 5-10 seconds keeping the Petri dish flat on the bench throughout.

6.4.5 Allow the agar to set, invert the Petri dishes and place them in the incubator.

6.5 Incubation and examination of the cultures

- 6.5.1 Incubate the two plates made with the highest dilution at 20-22 °C for three days. These extra plates allow for the higher counts expected at this temperature.
- 6.5.2 Incubate two of each of the remaining sets of plates at 37 °C for 24 ± 3 hours and two at 20-22 °C for three days.
- 6.5.3 Count the colonies as soon as the plates are removed from the incubator. If this is not possible, keep the plates at 4 °C for not longer than 24 hours.

- NB:*
- * Nutrient agar should be liquified in boiling water and cooled to 46 ± 2 °C before use. Before liquifying, visual inspection should be carried out to check whether nutrient media is free of secondary infection.
 - * Before pouring the nutrient media, the tube edge should be flamed. Only the culture plates not exceeding 300 colonies per ml should be used to enumerate the micro-organisms.
 - * If the colonies determined lie above 100, the figures are rounded off to complete tens, in the case of values over 1000, to complete hundreds and so on.
 - * It is a common practice to indicate the nutrient media used and the duration and the temperature of incubation in the analysis report.
 - * Take the average of the counts in each pair of plates and multiply the result by the dilution factor. If the number of colonies exceed 300 per ml, choose a dilution level in which the number of colonies lies between 30 and 300 and count the colonies in those plates.
 - * If the plate made with the highest dilution contains more than 300 colonies per ml, either try to count them and report the results as an approximate or express the count as more than 300n colony forming units per ml where n represents the dilution factor.

1. Application

1.1 The method is applicable to potable water, surface water and effluents.

2. Definition

2.1 In the context of the method, micro-organisms which produce acid from lactose and form yellow colonies on membranes after incubation for 4 hours at 30 °C followed by 14 hours at 37 °C are regarded as **presumptive coliform organisms**. Similarly micro-organisms which produce lactose after incubation for 4 hours at 30 °C followed by 14 hours at 44 °C are presumed to be **thermotolerant coliform organisms**. *E. coli* is a thermotolerant coliform organism which produces gas from lactose (or manitol) and which also produces indole from tryptophan.

3. Summary of method

3.1 Isolate the micro-organisms on a membrane placed on an absorbent pad saturated with a broth containing lactose and phenol red as an indicator of acidity and subsequently confirm the ability to produce gas and indole where necessary.

4. Apparatus

- 4.1 Membrane-filtration unit (Milipore)
- 4.2 Membrane filters (47 mm diameter; 0.45 µm pore size)
- 4.3 Absorbance pad (47 mm diameter; 1 mm thickness)
- 4.4 Incubator
- 4.5 Petri dishes
- 4.6 Pipettes (bacteriological)
- 4.7 Colony counter
- 4.8 Water bath
- 4.9 Autoclave

5. Reagents and media

- 5.1 **Membrane Lauryl Sulphate Broth (PHLS/SCA, 1980B)**
 - Peptone 40 g
 - Yeast Extract 6 g

Lactose	30 g
Phenol red (0.4% w/v aqueous solution)	50 ml
Sodium lauryl sulphate - specially pure	1 g
Distilled water	1 l

Add the ingredients to the water and mix gently to avoid froth. The final pH of the medium should be 7.4 to 7.5 and it may be necessary to adjust the pH to about 7.6 before sterilization to achieve this. Distribute in screw-capped bottles and autoclave at 115 °C for 10 minutes.

NB: * The media used with membrane filters differ in composition from those of the Multiple Tube Method and Pour Plate Culture Method because membranes selectively absorb some substances but not others.

6. Procedure

6.1 Preparation of the samples

- 6.1.1 The volumes should be chosen such that the number of colonies to be counted on the membrane lies, if possible, between 10 ml and 100 ml.
- 6.1.2 For treated waters, filter 100 ml of the sample.
- 6.1.3 For polluted waters, either filter small volumes or dilute the samples before filtration.

6.2 Filtration

- 6.2.1 Place the sterile filtration apparatus in position and connect to a source of vacuum.
- 6.2.2 Remove the funnel and place a sterile membrane (gird side upwards).
- 6.2.3 Replace the sterile funnel on the filter base.
- 6.2.4 Pour or pipette the required volume of water sample into the funnel.
- 6.2.5 Open the stop cock and apply the vacuum (500 mm Hg).
- 6.2.6 Close the stop cock as soon as the sample has been filtered.
- 6.2.7 Remove the funnel and transfer the membrane carefully either to a pad saturated with the medium or to a well dried agar plate (ensure that no air bubbles are trapped between the membrane and the medium).
- 6.2.8 Pour any excess of medium from the saturated pad either before or after the membrane is placed in position.

NB: * For different volumes of the same sample, the funnel may be re-used without boiling provided that the smallest volumes are filtered

first.

- * For different samples, remove a funnel from the boiling water bath, allow to cool and repeat the filtration procedure.
- * After filtration of each sample, disinfect the funnel by immersion in boiling distilled water for at least one minute.

6.3 Incubation and examination

- 6.3.1 Place the Petri dishes with the membrane inside a container with a tightly fitting lid to prevent drying out.
- 6.3.2 Incubate the membrane at the temperature for the duration of time specified for the organism sought.
- 6.3.3 After incubation count the characteristic colonies on the membrane within a few minutes under good light (if necessary use a hand lens).
- 6.3.4 Express the results as the number of indicator organisms per 100 ml of water sample.

- Advantages**
- * A quick method by which results can be obtained as direct counts.
 - * There is a considerable saving on labour, in the amount of media, and the glassware required.
 - * The conditions of incubation can be varied easily to encourage the growth of slow growing organisms.

- Limitations**
- * Gas production, for example, by coliform organisms is not detected.
 - * Membranes are not suitable for use with waters of high turbidity or those containing small numbers of indicator organisms sought.

7. Incubation and Examination of Membranes for Thermotolerant Coliforms and *E.coli*

- 7.1 Incubate the membranes for 4 hours at 30 °C and then for 14 hours at 44 ± 0.25 °C.
- 7.2 Count the yellow colonies within a few minutes of removal from the water bath or incubator.

- NB:**
- * Provided that the membranes are not overcrowded, colonies of *E.coli* usually have characteristic appearance of bright yellow in colour and are more than 1 mm in diameter.
 - * Total incubation period of 18 hours is recommended for the enumeration of coliform organisms and *E.coli*.

- * It is important to note that the counts of yellow colonies at 37 °C and 44 °C are only presumptive results and for potable water it is essential to carry out confirmative tests for coliform organisms and *E.coli* in order to assess the sanitary significance of the results.

8. Confirmation and Determination of Coliform Organisms

- 8.1 Subculture all yellow colonies or a representative number of them, in tubes of Lactose Pepton Water containing an inverted (Durham) tube in order to detect gas formation.
- 8.2 Incubate these tubes at 37 °C and examine them for the presence of acid and gas after 24 hours, and if the results are negative re-examine after a further period of 24 hours.
- 8.3 It is advantageous to subculture after 6 hours of incubation from the Lactose Peptone Water and plate on Nutrient-Agar and Mac Conkey-Agar to check the purity and colonial appearance.

9. Confirmation of *E.coli*

- 9.1 Subculture all yellow colonies or a representative number of them in tubes of Lactose Peptone Water and in tubes of Tryptone Water.
- 9.2 Incubate them at 44 °C for 24 hours and examine the tubes of Lactose Peptone water for the presence of acid and gas.
- 9.3 Add 0.2-0.3 ml of Kovac's reagent to the tubes of Tryptone Water. Development of red colour indicates the production of indole.

NB: * Yellow colonies on membranes incubated at 44 °C are regarded as *E. coli* if acid and gas are produced in Lactose Peptone Water and if the Indole Test is positive.

9.3.1 Tryptone Water for Indole Reaction

Certain peptones which give satisfactory results in tests at 37 °C are not satisfactory for the indole tests at 44 °C. Oxoid tryptone has been found satisfactory and is recommended.

Tryptone (Oxoid)	20 g
Sodium chloride	5 g
Distilled water	1 l

Dissolve the above ingredients in the water and adjust to pH 7.5. Distribute in 5 ml volumes and autoclave at 115 °C for 10 minutes.

9.3.2 Kovac's Reagent for Indole Test

Paradimethylaminobenzaldehyde	5 g
Amyl alcohol (analytical grade and free from organic bases)	75 ml
Hydrochloric acid (concentrated)	25 ml

Dissolve the aldehyde in alcohol. Add the concentrated acid with care. Protect from light and store at 4 °C.

(The reagent should be light yellow to light brown in colour; some samples of amyl alcohol are unsatisfactory, and give a dark colour with the aldehyde).

9.3.3 Lactose Peptone Water

Peptone	10 g
Sodium chloride	5 g
Lactose	10 g
Phenol red (0.4% w/v aqueous solution) (or Andrade's indicator)	2.5 ml (10 ml)
Distilled water	1 l

Dissolve the above ingredients in the water and adjust to pH 7.5. Add phenol red indicator and distribute in 5 ml volumes into tubes containing inverted fermentation (Durham) tubes. Alternatively, adjust to pH 6.8 to 7.0, and add the Andrade's indicator. Autoclave at 110 °C for 10 minutes. Alternatively, steam each sample for 20 minutes for three successive days. Test for sterility by incubation at 37 °C for 24 hours.

9.3.4 Mac Conkey Agar

Bile salts	5 g
Peptone	20 g
Lactose	10 g
Sodium chloride	5 g
Agar	12 g
Neutral red (1% w/v aqueous solution)	5 ml
Distilled water	1 l

Add the above ingredients to the water and steam to dissolve. Distribute into screw-capped bottles and autoclave at 115 °C for 10 minutes. For use, melt in steam and pour 15 ml into each sterile Petri dish.

9.3.5 Nutrient Agar

Nutrient broth gelled by the addition of agar

9.3.6 Nutrient Broth

Meat extract	10 g
Peptone	10 g
Sodium chloride	5 g
Distilled water	1 l

Add the above ingredients to the water and heat to dissolve. Adjust the pH to about 8.2 with a solution of sodium hydroxide and boil for 10 minutes. Clarify by filtration and adjust to pH 7.2-7.4. Dispense in bottles or tubes and autoclave at 115 °C for 10 minutes.

1. Application

1.1 The method is applicable to potable water, surface water and effluents.

2. Definition

2.1 In the context of the method, coliform organisms ferment lactose within 48 hours at 37 °C producing acids and gases. Thermotolerant coliform organisms (e.g. *E. coli*) show the same fermentative properties in 24 hours at 44 °C and produce indole from tryptophan.

3. Summary of method

3.1 Water samples or their dilutions are inoculated in a suitable broth (liquid nutrient medium). At the end of the incubation period, the tubes are examined for gas production by the coliform organisms. This test is known as a **Presumptive Test** since gas can also be produced by bacteria in addition to coliform organisms. The positive tubes of the presumptive test are subjected to a **Confirmatory Test** followed by a **Completed Test**.

4. Apparatus

- 4.1 Fermentation tubes
- 4.2 Inoculation loops
- 4.3 Water bath with a thermostat
- 4.4 Durham vials
- 4.5 Microscope
- 4.6 All the other basic materials required for total coliform counts

5. Reagents and culture media

- 5.1 Potassium dihydrogen phosphate [KH₂PO₄]
- 5.2 Sodium hydroxide [NaOH]
- 5.3 Magnesium chloride [MgCl₂]
- 5.4 **Mac Conkey's Broth** (single strength medium: pH = 7.4-7.5)
- 5.5 **Brilliant-green Lactose Bile Broth** (pH = 7.2)
 - Peptone 10.0 g
 - Lactose 10.0 g

	Ox bile (dehydrated)	20.0 g
	Brilliant-green	13.3 mg
	Distilled water	1 l
5.6	Eosine Methylene Blue (EMB) Agar (pH = 7.1-7.2)	
	Peptone	10.0 g
	Lactose	10.0 g
	Dipotassium hydrogen phosphate (K_2HPO_4)	2.0 g
	Agar	15.0 g
	Eosin Y	0.4 g
	Methylene blue	0.065 g
	Distilled water	1 l
5.7	Endo Agar (pH = 7.4)	
	Peptone	10.0 g
	Lactose	10.0 g
	Dipotassium hydrogen phosphate (K_2HPO_4)	3.5 g
	Agar	15.0 g
	Sodium sulphate	2.5 g
	Basic fuchsin	0.5 g
	Distilled water	1 l

6. Procedure

6.1 Presumptive Test

- 6.1.1 Select the dilution according to the expected bacterial count using Table 5.2.
- 6.1.2 Select the number of tubes for each sample as given in Table 5.2. Put Durham vials inverted in each test tube and put the media required. Sterilize the tubes at 121 °C for 15 minutes.
- 6.1.3 Shake all the water samples vigorously immediately before removing sample aliquots to inoculate the series of test tubes.
- 6.1.4 Add samples using sterilized pipettes to the test tubes selected for the test and mix thoroughly. Use separate pipettes for different samples as well as for dilutions. While withdrawing sample portions, the tip of the pipette should never be submerged more than 1 inch below the surface of the sample. This procedure minimizes the accumulative drainage from exterior of pipette into the media.
- 6.1.5 Place within 30 minutes, all these tubes in an incubator at 35-37 °C.
- 6.1.6 After 48 hours, examine each tube carefully. Those showing gas

in the Durham vials are recorded as positive (+). Gas in any quantity even a tiny bubble is recorded as (+). The tubes showing **Positive Test** are subjected to **Confirmatory Test**, as gas production is not the only criterion for a positive test. Discard all the Durham tubes first at the completion of 24 hours. Subject the tubes showing positive test immediately to a confirmatory test. Incubate negative tubes to a further period of 24 hours and observe any positive results.

Table 5.2 Dilution of water samples for the Presumptive Test

Type of water	Sample volume (ml)	Culture volume (ml)	No. of tubes
Potable water	10	10 (double strength)	05
Surface water (unpolluted)	10	10 (double strength)	05
	01	10 (single strength)	05
	0.1	10 (single strength)	05
	0.01	10 (single strength)	05
Polluted with domestic sewage	0.1	10 (single strength)	05
	0.01	10 (single strength)	05
	0.001	10 (single strength)	05
	0.0001	10 (single strength)	05

6.2 Confirmatory Test

- 6.2.1 For this test, Brilliant-green Lactose Bile Broth (BGLB) is used.
- 6.2.2 Prepare fermentation tubes with 10 ml BGLB medium and put Durham vials inverted in each tube. The number of tubes to be prepared is equal to all positive tests in the presumptive tests.
- 6.2.3 Shake gently, the fermentation tubes of presumptive test with positive results and transfer one loop-full of medium to BGLB broth.
- 6.2.4 Incubate the tubes at 35-37 °C for 48 ± 2 hours and record the tubes with gas formation as positive. Remember that wrong concentration of BGLB or the exposure of the media to excessive heat or light may give false positive tests.

6.3 Completed Test

- 6.3.1 Since some of the positive results from the Confirmatory Test may be false, it is desirable to repeat the completed test occasionally.

For this, inoculum from each positive tube of the confirmatory test is streaked on a plate of Eosine Methylene Blue (EMB) or Endo Agar.

- 6.3.2 Prepare Endo Agar (5.7) or EMB (5.6) Agar Petri dishes. The number of Petri dishes to be prepared is the same as that of tubes showing gas production in BGLB medium. Label the dishes with the corresponding numbers of the tubes of confirmatory test.
- 6.3.3 Streak inoculum of the BGLB tubes on the Petri dishes in such a way that the colonies after separation have a distance of 0.5 cm.
- 6.3.4 Incubate these Petri dishes at 37 ± 2 °C for 24 hours.
- 6.3.5 Now examine the dishes for bacterial growth and colony appearance. Well isolated colonies with a dark centre (nucleated) are the typical coliform colonies. They may have a metallic surface sheen. The colonies that are pink or opaque are not nucleated. They are typical colonies and may belong to the coliform group. Clear, watery colonies are not of the coliform group and are reported as negative in the completed test.
- 6.3.6 Now inoculate a coliform colony isolated (avoid picking a mixture of colonies) from each place into the tubes of MacConkey's broth and record the gas production (a repetition of the presumptive test but with the colonies) within 48 hours at 37 °C.
- 6.3.7 Also examine the colonies by **Gram Staining**. For this, transfer the colonies to a nutrient agar slant. Subject the colonies obtained from agar slants to the Gram Staining.
- 6.3.8 If organisms appear rod (bacilli) shaped, red stained and occurring single or in pairs or in short chains, the test is confirmed. Since the coliform organisms are Gram-negative, if the Gram-positive organisms or spore forming organisms appear, this portion (completed) of the test is noted as negative.

1. **Application**

1.1 The method is applicable to potable water, surface water and effluents.

2. **Definition**

2.1 Thermotolerant coliform bacteria, especially *E. coli* derived from human excreta ferment Lactose within 24 hours at 44 °C. *E. coli* also produces indole from tryptophan.

3. **Summary of method**

3.1 Conduct the presumptive test in the same way as the total coliform count but the confirmatory test should be carried out as outlined below.

4. **Apparatus**

4.1 Fermentation tubes

4.2 Inoculation loops

4.3 Durham vials

4.4 Water bath with a thermostat

4.5 Microscope

4.6 All other materials required for total coliform count

5. **Reagents and culture media**

5.1 Potassium dihydrogen phosphate [KH₂PO₄]

5.2 Sodium hydroxide [NaOH]

5.3 Magnesium chloride [MgCl₂]

5.4 **Mac Conkey's Broth** (single strength medium: pH = 7.4-7.5)

Bile salts (Sodium taurocholate) 5 g

Peptone 20 g

Lactose 10 g

Sodium chloride 5 g

Bromocresol purple (1% ethanolic solution)
or 1% neutral methyl red 1 ml

Distilled water 1 l

5.5 *E. coli* medium

5.5.1 **Lauryl Tryptose (Lactose) Broth** (double strength)

Tryptose	40 g
Lactose	10 g
Sodium chloride	10 g
Di-potassium hydrogen phosphate	5.5 g
Potassium dihydrogen phosphate	5.5 g
Sodium lauryl sulphate - specially pure	0.2 g
Distilled water	1 l

Add the tryptose, sodium chloride, lactose and phosphates to the water and warm to dissolve. Add the sodium lauryl sulphate and mix gently to avoid froth. Adjust to pH 6.8. Prepare single strength medium by dilution of the double strength medium with an equal volume of distilled water.

Distill single strength medium in 5 ml volumes and double strength medium in 10 ml and 50 ml volumes. Each tube or bottle should contain an inverted fermentation tube. Autoclave at 115 °C for 10 minutes.

6. **Procedure**

6.1 **Presumptive Test**

6.1.1 Perform presumptive test as of the total coliform count.

6.2 **Confirmatory test**

6.2.1 Carry out the test exactly as of the confirmatory test for total coliform count but use *E. coli* medium instead of BGLB and incubate the tubes at 44.5 °C in a water bath within 30 minutes after inoculation.

6.2.2 Remove the tubes after 24 hours.

6.2.3 Shake the tubes gently and observe the gas production. The test is positive if any gas is produced. Such tubes may be subjected to the completed test.

7. **Calculation**

7.1 Calculation of Most Probable Numbers (MPN)

7.1.1 The calculation of MPN of coliform organisms is done by combination of positive and negative results in the Multiple Tube Test. The values can be calculated for any of the combinations

given in Table 5.3. Refer Table 5.4 for estimation of Most Probable Number (MPN), if the tubes of only one sample portion (10 ml) have been used, as is usually done for potable waters. If three combinations, e.g., 10 ml, 1 ml and 0.1 ml have been used, refer Table 5.3. The important thing to remember is that the positive and negative combinations of any one test can be used e.g., if a test has been carried out only upto the presumptive test stage, than the positive and negative combinations of this test can be used to calculate the MPN. If all three tests had been carried out, the MPN can be calculated on the basis of either presumptive, confirmatory or completed test.

7.1.2 For example, let us assume that 5 tubes each of 10 ml, 1 ml and 0.1 ml sample portions were used and the results of the test are as follows:

- 10 ml portion - 2 tubes positive, 3 tubes negative
- 1 ml portion - 2 tubes positive, 3 tubes negative
- 0.1 ml portion - All tubes negative

7.1.3 The combination can be written as 2-2-0 and the MPN index according to the Table 5.3 will be 9 cell per 100 ml. If a combination other than those given in the table appears, then perhaps the test has not been carried out according to the instructions.

7.1.4 The Table 5.3 is given for starting dilution of 10 ml, 1 ml and 0.1ml. If any other dilution pattern is used (e.g., 100 ml, 10 ml and 1 ml), the same table can be used, but here, 100, 10 and 1 will be supposed as 10, 1 and 0.1, and the MPN obtained from the table will be put in the following formula to get the actual MPN per 100 ml of the sample:

$$\text{MPN per 100 ml} = \frac{\text{MPN table value} \times 10}{\text{Starting dilution}}$$

7.1.5 For example, if the results are as same as previously given 2-2-0, but the dilutions used are 100 ml, 10 ml and 1 ml, then according to the formula,

$$\text{MPN per 100 ml} = \frac{9 \times 10}{100} = 0.9$$

7.1.6 If more than three dilutions are used, then select three successive dilutions for the calculation of MPN, starting from the highest dilution (the lowest sample fraction) which gives positive results in all five tubes and next two lower dilutions.

7.1.7 In case of unlikely combinations, use the following formula to calculate MPN per 100 ml:

$$\text{MPN per 100 ml} = \frac{\text{No. of positive tubes} \times 100}{\text{Total sample (ml) in negative tubes}} \times \text{total sample in test}$$

These tables can be similarly used for faecal coliforms.

Table 5.3 MPN per 100 ml for various combinations of positive results when 5 tubes each of 10, 1 and 0.1 ml sample fractions are used

Combination	MPN per 100 ml	Combination	MPN per 100 ml
0-0-0	2	4-3-0	27
0-0-1	2	4-3-1	33
0-1-0	2	4-4-0	34
0-2-0	4	5-0-0	23
1-0-0	2	5-0-1	31
1-0-1	4	5-0-2	43
1-1-0	4	5-1-0	33
1-1-1	6	5-1-1	46
1-2-0	6	2-1-2	63
2-0-0	5	5-2-0	49
2-0-1	7	5-2-1	70
2-1-0	7	5-2-2	94
2-1-1	5	5-3-0	79
2-2-0	9	5-3-1	110
2-3-0	12	5-3-2	140
3-0-0	8	5-3-3	180
3-0-1	11	5-4-0	130
3-1-0	11	5-4-1	170
3-1-1	14	5-4-2	220
3-2-0	14	5-4-3	280
3-2-1	17	5-4-4	350
4-0-0	13	5-5-0	240
4-0-1	17	5-5-1	350
4-1-0	17	5-5-2	540
4-1-1	21	5-5-3	920
4-1-2	26	5-5-4	1600
4-2-0	22	5-5-5	2400
4-2-1	26		

Table 5.4 MPN per 100 ml values when five tubes of only 10 ml are used

No of tubes giving positive results out of five	MPN per 10 ml
0	0
1	2.2
2	5.1
3	9.2
4	16.0
5	16.0

CHAPTER 6: LABORATORY ORGANIZATION, DATA PROCESSING, QUALITY CONTROL AND DISSEMINATION

6.1 Laboratory Organization

Water laboratories must be organized or set up to meet the objectives of each assessment programme. Attention should be paid mainly to the choice of analytical methods. In many countries monitoring laboratories are organized in two ways: (1) regional laboratories to conduct basic determination not requiring very complex equipment, and (2) central laboratories to conduct more complex analysis requiring sophisticated equipment and well trained personnel. In addition, central laboratories often provide the regional laboratories with methodologies and analytical data on quality control. Compatibility of water quality data from different laboratories can only be ensured if identical or at least, similar methods are used.

Progress in analytical chemistry has stimulated the appearance of new, more advanced and more efficient methods. However, replacement of existing methods often results in data compatibility with older methods which can cause problems in time series analysis. Studies on the compatibility of new methods with old methods must be undertaken. If new methods are accepted into a monitoring programme an overlap period is required where samples are analyzed by both the new and old methods.

6.2 Data Processing

Analytical data collected by laboratories, together with the information on sampling and hydrological parameters are usually sent to a data processing centre which is not found in our country. However, it is reasonable to have a common information centre which compiles the data from the entire country. The main objective of a data processing centre is the development, replenishment and management of the data bank. At the initial stage of development of a data bank, attention should be focused principally on the accuracy of the stored information because the forms of output may be improved in the process of data bank exploitation.

6.3 Quality Control Programme

Quality Control (QC) is the process of monitoring laboratory analysis to ensure results of known and defensible quality. A QC programme monitors test performance, helps to identify problems with a specific analysis, and assists scientists in assessing the reliability of results. Quality Control Programme is

generally confined to a particular laboratory. Experts agree that 10 to 20 % of resources including man power should be directed towards ensuring the quality of analytical data. When trace pollutants (e.g. pesticides and heavy metals) are measured the resources required for quality control may reach 50 %. Unfortunately, specially in developing countries this problem is not given adequate attention. This results in the generation of unreliable data and hence, unsatisfactory solutions to the water quality problems.

However, it is necessary for an analytical laboratory to be furnished with the following so as to perform high quality analysis:

- Inter-laboratory calibration test
- Well equipped laboratories with technical accessories
- Adequate conditions for maintenance of analytical instruments
- Reliable and steady supply of laboratory reagents, solvents, gases of specified grade and standard samples
- A systematic quality control programme

Control samples with known values are provided with control values and \pm standard deviation range. This information allows an individual laboratory to compare its data. The data may help in identifying a particular problem with an analysis or may indicate a better method for a procedure that is to be modified.

A good QC programme consists of at least 5 elementary requirements for the following tests to be done regularly to ensure the reliability of data:

1. Assessment of analyst competence
2. Reagent blank check
3. Calibration with standards
4. Duplicate analysis
5. Errors and control charts

6.3.1 Assessment of Analyst Competence

It is very important to be conversant with a particular analytical method before proceeding with the real analysis. Follow the procedure given below to self-check your ability.

- Select the water quality parameter that you intend to use in the assessment of analyst competence.
- Make a minimum of four replicate analysis of independently prepared check samples having a concentration between 5 and 50 times as the

- method detection limit for the analysis in the laboratory
- Determine the concentration of replicates and record the results to calculate the precision of analysis.
- Compare your results with the following chart (Table 6.1). If the results do not give an acceptable value, get the confidence of the method before proceeding further.

Table 6.1 Precision of analytical results of different parameters

Analysis	Precision (%) Concentration Duplicates < 20xD.L	Precision (%) Concentration Duplicates > 20xD.L
Metals	75 - 125	90 - 110
Anions	75 - 125	90 - 110
Nutrients	75 - 125	90 - 110
TOC	75 - 125	90 - 110
Microbial	75 - 125	90 - 110

D.L. - Detection limit

6.3.2 Reagent Blank Check

The purity of reagents plays an important role in the final results. Follow the guidelines of chemical grade when selecting chemicals to prepare reagents for a particular analysis.

- Always analyse a minimum of 5% of the number of samples as reagent blanks.
- Analyse a reagent blank after any sample with a concentration more than that of the highest standard to avoid carryover from one sample to another.

6.3.3 Calibration with standards

- Always use four standards to plot a calibration curve when an analysis is initiated.
- Verify the validity of the calibration curve by analyzing two standards within the linear range before using the previous calibration curve again. If the points are not on the calibration curve, prepare a fresh calibration curve.

- Do not report the values above the highest standard used in the calibration curve. Do a dilution to bring the concentration of the sample down to the linear range of calibration curve.
- The lowest reportable value is the detection limit of the method.
- If a blank is subtracted, report the result even if it is negative.

6.3.4 Duplicate Analysis

In order to assess the precision, duplicate analysis have to be performed. Always analyze 5% of the samples in duplicate, calculate the % of precision. Check the value with the Table 6.1 for acceptable results of duplicate analysis.

6.3.5 Errors and Control Charts

Two types of errors may be encountered during the analytical process random and systematic.

Random Errors

A random error is one with no trend or means of predicting it. Frequently occurring random errors include:

- Mislabelling a sample
- Pipetting errors
- Improper mixing of sample with reagents
- Voltage fluctuations not compensated by the instrument electronics
- Temperature fluctuations.

Systematic Errors

A systematic error will be seen as a trend in data. Control values gradually rise (or fall) from the previously established limits. This type of error include:

- Improper calibration
- Deterioration of reagents
- Sample instability
- Instrument drifts

Control Charts

Both random and systematic errors can be identified and corrected by maintaining control charts. The values should be taken from the control samples.

Construction of Control Charts (figure 6.1)

- Obtain the mean and standard deviations of control solution. Usually the mean and standard deviations of the control solution are provided from the Central Monitoring Laboratory.
- Plot the following lines as Y- co-ordinates:
 - Line 1: Mean (\bar{x})
 - Line 2: Warning limits (WL) [$\text{mean} \pm 3s$]
 - Line 3: Control limits (CL) [$\text{mean} \pm 2s$]
- Plot the date analyzed as X- co-ordinates.
- Prepare a separate control chart for each water quality parameter, and display it in the lab.
- When conducting routine analysis, always include a sample of control solution in the batch.
- Plot the results of control solution in the respective control chart before proceeding with further analysis.

NB: * Widely scattered points indicate low precision,
* Note systematic changes in calibration curve.

Check Points (figure 6.1)

Control limit (CL): If one measurement exceeds CL, repeat the analysis immediately. If the new result is within the CL, continue the analysis. If it exceeds stop the analysis and correct the problem.

Mean line (ML): If six successive samples are above the ML, analyze another sample. If the next point is below the ML, continue the analysis. If the next point is on the same side, discontinue the analysis and correct the problem.

Warn limit (WL): If two out of three successive points exceed a WL, analyze another sample. If the next point is less than WL, continue the analysis. If the next point exceeds WL, discontinue the analysis and correct the problem.

NB: * Always prepare a control solution of concentration near the upper limit of the calibration curve.

6.4 Quality Assurance Programme

A good quality control programme is a part of the overall goal of quality assurance [QA], which encompasses every aspect of the laboratory operation from sample collection and preservation to the clear reporting of the final laboratory

results. Therefore quality assurance is intricated with the total process, not simply the results produced in the laboratory. The data generated following the quality control procedures will be utilized in data auditing process usually by the central laboratory. Therefore, care should be taken to maintain the laboratory according to the following guidelines.

- Quality assurance plan with authorization
- Calibration graphs with recording facilities of occasional calibration points
- Laboratory mean control charts
- Quick guide of analytical methods

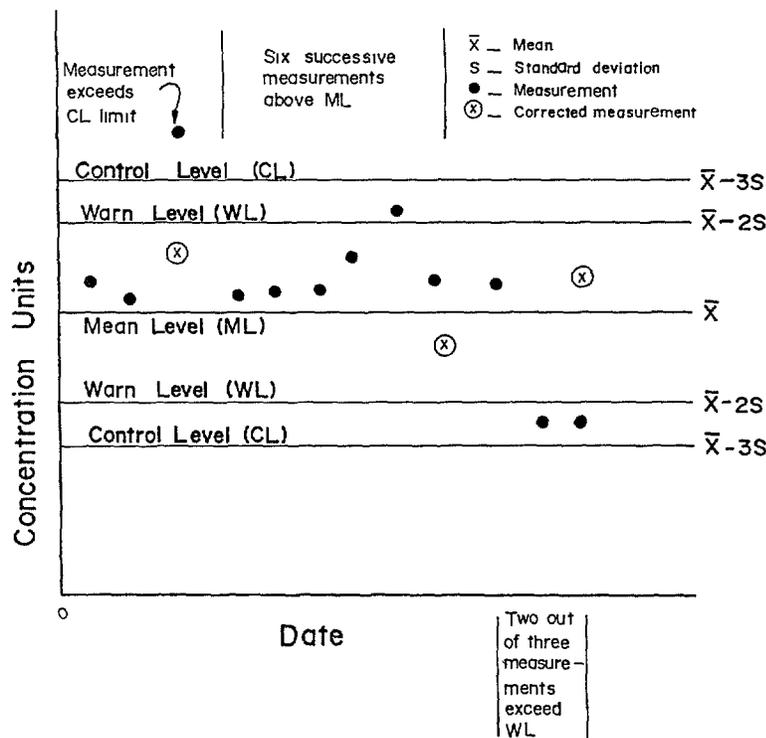


Figure 6.1 Control Chart

- Quick guide of the operation/maintenance of equipment
- A laboratory copy of the operating manual and detection limit chart of all parameters analyzing in the laboratory
- Status report of purity of reagents, chemicals, etc.
- Quality of glassware and other laboratoryware
- Waste disposal procedures
- Unified data coding sheets
- Laboratory safety procedures
- Laboratory book/data recording

If this information is not in order, make immediate arrangements to have the appropriate documents ready prior to routine analysis in the laboratory.

6.5 Interpretation and Dissemination of Data

Interpretation of results and dissemination of data are the final two steps in an assessment programme. Correctly, interpreted results will not be of much use if they are not disseminated to all relevant authorities, scientists and the public in a form which is readily understandable and acceptable to the target audience. The form and level of data presentation are therefore crucial. Usually, the interpretation of results is undertaken by subject specialists. Interpretations should be always referred to the objectives and should also be proposed improvements, including simplifications, in the monitoring activities as well as the need for further research and guidelines for environmental planning and economic development. Subsequently, these findings should be discussed with the appropriate local, regional and national authorities and, others such as the industrial development and/or national planning bureaus as required. Beside these authorities, results should be communicated to water resource managers, the public associations for environmental protection, educational institutions, other countries and to relevant international organizations.

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APPENDIX UNITS AND CONCENTRATIONS

Equivalence of units

Quantity	Old Style	SI system
Volume	ml	dm ³ cm ³
Chemical amount	eq (substance from context)	mol (substance specified e.g. Na ⁺ , ½Ca ²⁺)
Mass concentration	μg ml ⁻¹ = mg l ⁻¹ = ppm = gm ⁻³	μg cm ⁻³ = mg dm ⁻³ = gm ⁻³
Chemical concentration	N = eq l ⁻¹ (substance from context) meq l ⁻¹	mol dm ⁻³ (substance specified) mmol dm ⁻³

Expressions of concentration

Expression	Definition	Symbol	Units
Molarity	Moles of solute ----- Liter solution	M	mol l ⁻¹
Molality	Moles of solute ----- Kilogram solvent	m	mol kg ⁻¹
Normality	n [*] x molarity	N	mol l ⁻¹
Weight %	Mass of solute ----- x 100 Total mass of the solution	wt/wt %	---
Parts per thousand	Grams of substance ----- x 10 ³ Grams of sample	ppt	---
Parts per million	Grams of substance ----- x 10 ⁶ Grams of sample	ppm	---
Parts per billion	Grams of substance ----- x 10 ⁹ Grams of sample	ppb	---

* n is the number of protons donated or accepted by the species

Concentrated acids and bases

Reagent	Approximate weight %	Approximate molarity	ml of reagent needed to prepare 1L of ≈ 1.0M solution
Acetic (CH ₃ CO ₂ H)	99.8	17.4	57.5
Hydrochloric (HCl)	37.2	12.1	82.6
Hydrofluoric (HF)	49.0	28.9	34.6
Nitric (HNO ₃)	70.4	15.9	62.9
Perchloric (HClO ₄)	70.5	11.7	85.5
Phosphoric (H ₃ PO ₄)	85.5	14.8	67.6
Sulfuric (H ₂ SO ₄)	96.0	18.0	55.6
Ammonia (NH ₄ OH)	28.0	14.5	69.0
Sodium hydroxide (NaOH)	50.5	19.4	51.5
Potassium hydroxide (KOH)	45.0	11.7	85.5

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